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Micro-nutrient elements—a recapitulation

H. D. KAY

Introduction

Very material advances have been made in the last few years in our knowledge of the occurrence in living tissues and of the biological functions of the so-called micro-nutrient elements. The object of the present article is to bring to the attention of those food technologists who may have had little time to keep up with the large volume of recent, and fairly recent, publications in this field a selection of these advances, especially those with a bearing on the supply and quality of human and animal food. In the scope of some 12,000 words the selection of significant topics from this vast and growing field must inevitably be both limited and arbitrary.

Definitions

'Micro-nutrient' and 'trace element' are terms that are frequently used in the food science field, but both, for present purposes, require some semantic attention. The term 'trace element' is obviously intended to indicate an element in some form of combination in living tissue in an amount that is quite small relative to the total quantity of the main tissue constituents, but 'trace', 'micro' or 'quite small' are not always easy to define. Analytically speaking one chemist's 'trace' may be another chemist's abundance. Can we, therefore, set a numerical limit above which 'trace' or 'micro' cease to apply? Is iron a trace element in this context? Difficulties arise because what some may regard as a trace element may be present in different tissues of the same animal in quantities that vary very widely. Iron in the erythrocyte (say 2500–3000 ppm on the dry weight) or copper in the normal ruminant liver (200–1200 ppm on the dry weight) are present in amounts that many would not regard as 'trace' or 'micro' quantities. If we use the more precise term 'essential micro-nutrient', i.e. a substance necessary in very small amounts for the healthy functioning of a living organism, plant or animal, including, in the animal, functions such as 'normal' growth ('normal' is another word difficult to define), skeletal and tooth development and maintenance, locomotion, blood formation, hair growth and pigmentation, reproduction and milk production, and analogous functions in the plant, then we include along with the trace elements, the vitamins. A still further limitation is necessary. It is the essential 'mineral' micro-nutrient elements with which the present article is concerned, and iron will be excluded from these.

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The matter of essentiality is given more detailed consideration by Bowen (1966, p. 102 *et seq.*).

What, then, is the present roster of these elements? In view of the wide distribution in the earth's crust and the oceans of all the elements in the periodic table, and not forgetting Avogadro's number, it is virtually certain that practically every one of these elements with the possible exception of the trans-uranium ones could be detected in the tissues and body fluids of every large organism if our methods of analysis were sufficiently delicate. In fact the majority of these elements have been so detected (Bowen, 1966, pp. 70 and 71) in animal and plant tissues. It is a little surprising, nevertheless, to know that we have uranium in our tissues and excrete it at the rate of 0·005–0·007 ppm in our urine (Wing, 1965) and gold in our hearts (Wester, 1965) but only in miniscule quantities!

The list of essential mineral micro-nutrients that are now well authenticated is shown in Table 1. The possibility must not be ruled out that, amongst the trace elements that

TABLE 1. Essential micro-nutrient elements

For man and other higher animals	For higher plants
Co, Cu, Mn, Mo, Zn	Cu, Fe, Mn, Mo, Zn
Se, F, I	B, Cl, Si
Present in many tissues in micro quantities, but probably not essential	
Al, As, Br, Cd, Cr, Rb, Si, Sr	
Found in certain lower animals, but function dubious	
V, Nb, Ti, Ta	

Notes: Fe has not been included in the animal list, for reasons given in the text; B is not required by animals; Cl is essential in small quantities for most plants; Si is essential for most grasses and many other plants.

are present regularly in normal animal or plant tissue (such elements as As, Ba, Br, Cd, Cr, Rb, Si and Sr) in amounts comparable with those of the authenticated list shown in Table 1, one or more will eventually graduate into the essential category. Perhaps the most likely candidate at present for such promotion is chromium, which in some animal organs (e.g. the bovine liver) is closely associated with nucleoprotein. It has been found in apparently significant quantities in various RNA concentrates and may have a specific function as a constituent of a 'glucose tolerance factor' of importance in the metabolism of the rat and possibly other animals. All these candidate elements occur in easily or fairly easily detectable amount in soils, plant tissues and ordinary mixed human diets, and some (e.g. Cd, Rb, Si and Ba) though not known to form an essential part of an enzyme molecule, are reported as having an adjuvant function in relation to one or more plant or animal enzymes.

With the exception of fluorine and selenium, all the 'authentic' essential trace

elements shown on the left in Table 1 are present in animal and in human tissues as an integral part either of an enzyme, a vitamin or a hormone. An enzyme being a protein with an unusual strain in the molecule, the presence in a protein of a trace metal, particularly one with a variable valency, though not *ipso facto* indicating that the protein has enzymic properties, at least provides one of the requirements for potential enzyme activity. In carefully controlled experiments with laboratory animals, usually rats or other mammals, and with farm livestock, each of these authentics has been shown to be necessary for health. Though such critical, controlled experiments can hardly be performed on man, it seems even more admissible in the case of the trace elements than it is for the vitamins, to regard, without too serious misgivings, the results of such animal experiments as applicable to man. Nevertheless, in a few animal species, certain rather unexpected trace elements that do not appear necessary for other organisms are regarded as probably playing a vital part in their metabolism. Such for example is vanadium, which was shown as long ago as 1911 to be present in quantities which, from the trace-element standpoint, must be regarded as substantial, in the greenish circulating blood cells of certain tunicates as a vanadium–protein compound. It would perhaps be biochemically tidy if the vanadium, which, like iron in haemoglobin or copper in the haemocyanin circulating in the blood of certain crustaceans (referred to later) has a variable valency, acted as part of a respiratory pigment for these tunicates, but this does not appear to be the case. These animals presumably pick up their vanadium preferentially by some discriminative mechanism not yet explained, from the very small amounts present in sea water. Members of this sub-phylum of invertebrates appear to be a little catholic in their taste for unusual trace elements; niobium, titanium and tantalum are said to be present in some of the species (Kokubu & Hidake, 1965), or even in individuals within a species. Vanadium occurs in *very* small, but not vanishingly small, amounts in plants and in mammalian tissues, including red cells, and it has been suggested that it may possibly have significance in relation to cholesterol synthesis or to atherosclerosis [Underwood (1962, p. 353) for discussion of this and other possible medical relationships of this metal].

Whilst for the higher animals including, presumably, man, normal growth, health and reproduction are dependent on there being a sufficiency in the diet of all the elements shown at the top of the left column in Table 1, several of these become toxic if this sufficiency is greatly, or in the case of Se and Mo not very greatly exceeded. Copper is one of the less toxic, though rather more toxic for ruminants than for monogastric animals, and up to 1000 ppm or even more may be found in the liver of a normally functioning animal that has eaten a diet containing above the average amount of this trace element. Zinc, similarly, may be found in the organs and tissues of apparently entirely normal animals in surprisingly large amounts for a 'trace' element.

Though from the early days of analytical chemistry the investigation of the identity, quantity and possible function of several of the micro-nutrient elements in soils, in plants and in animal tissues has been fairly actively pursued, it has never been an easy

task. Even today, in spite of a keener realization of the possibilities of local contamination, of the increasing purity of analytical reagents, of the steady improvement in microchemical techniques, of the highly refined physical approaches such as the various modern spectroscopic techniques, radioactivation methods, etc., there is still considerable room for error in the determination of what are usually quite tiny quantities—a few parts per million or even fractions of parts per million—distributed in a relatively vast mass of stubborn tissue. Results obtained in different laboratories for the amount of the same trace element in the same tissue may still disagree disturbingly. Nevertheless, there can be no doubt that in general the accuracy and reproducibility of the quantitative information now available—and, therefore, the value of our deductions as to the detailed functions and interrelationships of the essential trace elements—have greatly increased in the past two or three decades. The economic return from the practical application of this knowledge has risen and is still rising, particularly in relation to crop production and animal husbandry.

These happy developments have been accompanied by a deluge of original papers, monographs, reviews and books, and within the limits of this article it would be impossible to cover more than a small fraction of the findings so voluminously dealt with or to provide a detailed bibliography. (A list will be found at the end of the article of several sources in which extended bibliographies, covering nearly all of the fairly recent literature, are provided.)

Except for iodine and fluorine, and probably selenium, the relationship between the micro-nutrients named in Table 1, and *human* growth and health has received only limited attention (Table 2 has relevance here). This is partly because of the difficulties of acute nutritional experiments with man, and partly because it is inherently unlikely that a human being, on any of the types of mixed diet that are customary at any rate in 'developed' countries, will suffer either from deficiency or excess of Co, Cu, Mn, Mo or Zn, (though toxic symptoms have occasionally been reported when individuals have been occupationally over-exposed). With regard to infants and other humans on restricted diets, or in populations in under-developed countries where the food quality or quantity may be grossly unsatisfactory this statement may not apply. Since, as a broad generalization, the content in protein-poor foods of the essential trace elements is smaller than in foods adequate in protein, children in under-developed countries, many of whom are either suffering from overt protein deficiency or are on the border line of protein insufficiency, may also be short of trace elements as well. As regards farm animals, trace element deficiency is still far from infrequent in several parts of the world, even in the U.K. and other agriculturally knowledgeable countries.

Where, in practice, the micro-nutrient trace elements not infrequently affect human nutrition and health is of course *indirectly* through the deleterious effects of deficiencies or excesses of *plant* micro-nutrients in the soil. These diminish the yield per acre and frequently the quality of crops such as cereals, potatoes, and other vegetables and horticultural produce used for immediate human consumption. At one further remove,

TABLE 2. Sources in human diet, approximate (presumptive) daily requirement for a human adult, and safety margins of the essential micro-nutrients

Micro-nutrient and atomic weight	Probable average daily dietary requirement	Margin of safety high ≡ >20	Food sources	
			Good	Fairly good
Co (59)	Less than 0·1 mg (as vitamin B_{12})	Fairly high	Liver, kidney, oysters	Meats
Cu (63·5)	1–3 mg	High	Ruminant liver and kidney, oysters and other shell-fish, crab, lobster, nuts	Leafy vegetables, peas and beans, meats, whole meal bread
Mn (55)	3–5 mg	High	Peas and beans, nuts, cereals, tea	Leafy vegetables fruits
Mo (96)	Very small	Fairly high	Peas and beans	Legumes, cereal grains, liver, kidney
Zn (65·4)	10–20 mg	Very high	Oysters, wheat germ	Kidney, liver most high-protein foods
Se (79)	Very small (? 0·1 ppm of diet)	>10	Present in small quantities in many foods and in sufficient quantity in a normal mixed diet	
F (19)	1–2 mg	To tooth mottling about 3; but much higher levels tolerated	Tea, mackerel, salmon, sardines	Kidney, liver, wheat germ
I (127)	0·06–0·15 mg	High	Sea fish, fish oils, shell-fish, seaweed, (iodized salt)	Yolk of egg
Fe (56)	12–15 mg	High	Meat, liver, egg-yolk, whole wheat, beans, spinach	Prunes, raisins, green vegetables

Note 1. All but one (Zn) of the *metallic* micro-nutrients are transition metals of variable valency; all but one have atomic weights between 55 and 65·4; all without exception readily link with soluble proteins.

Note 2. Deficiency of any of the above micro-nutrients, except I and F, is probably rare in most western or mixed human diets, though sub-clinical deficiencies may possibly occur, and certain individuals may have unusually high requirements. In most of the food sources of micro-nutrients the micro-nutrient status of the soil, or of the diet of the animal is an important factor.

where crops used for the main feed of farm animals are scanty or defective because of soil deficiencies in micro-nutrients, the output from these animals of such products as milk, eggs or wool, their reproductive ability and their ultimate value as human food will all diminish.

It is essential, at this point, to go a little further into the soil–plant–animal–human relationships of the micro-nutrients.

Deficiencies in the plant *macro*-nutrients N, P, K and Mg, often associated with the soil's lime status or with the soil pH have for many years been studiously corrected by the majority of competent farmers or horticulturists. Shortages or imbalance of the *micro*-nutrients, which can in certain circumstances be no less grievous than lack or imbalance of the well-established fertilizers, have received serious attention only during the last 25–30 years. Even today, in several agriculturally progressive countries, lack of knowledge of the requirements of crops in many farming areas for micro-nutrient elements is widespread, usually because soil analytical data are scanty. In most of the less developed countries ignorance of the micro-nutrients is almost universal and analytical data hardly exist, though in a limited number of these countries a start has been made (e.g. Wahab & Bhatti, 1958).

To quote history, it was accidentally discovered by a perceptive farm manager in Brandenburg in the early 1900s that dressings of copper sulphate on certain moorland soils and ploughed-up pastures markedly improved the yield and quality of cereals, peas, beans and lupins (Perkow, 1966). A mustard-infested oat field which it was intended to spray with ferrous sulphate was, by the mistake of a farm worker, sprayed instead with copper sulphate. The remarkably healthy appearance and unexpectedly large yield of oats in this field as compared with the neighbouring fields, together with the yield of the bean crop grown on the sprayed area in the following year, led the observant manager not merely to a discovery of the mistake but also to systematic tests of the effects of copper on other species of cultivated plants.

As regards animals, the story of the findings in New Zealand and Australia, beginning some 30 years ago, that certain dystrophies of farm stock living entirely on locally-produced grass or other locally-grown feeds ('bush sickness', 'phalaris staggers', 'peat scours', 'falling disease', anaemias, etc.) and also that serious faults in wool yield and quality could be prevented by treatment of the animal or the soil with small amounts of cobalt and/or copper is now well known (Marston, 1935). It had also been shown that for young piglets being raised in concrete sties, small dietary additions of copper, as well as iron, were highly desirable.

Modern soil surveys in the more developed countries, particularly in areas where deficiency symptoms in plants or animals are suspected, have of recent years often included, in addition to determinations of the *macro*-nutrients, analyses and estimations of the probable availability to the plant* of some at least of the micro-nutrients essential

* A recommended method for copper (Patterson, 1965) is extraction of the soil with 0·05 M concentration of the ammonium salt of EDTA at pH 4.

for its healthy development. In some cases these analyses extend to other trace elements which, though not proved essential for the plant, are known to affect animal health and are normally conveyed through the plant to the grazing animal (Co, F, Se and I). Such work has shown that serious soil deficiencies in trace elements essential for plant and/or animal health are widespread, not merely in Australia and New Zealand, but also in Western Europe (including parts of the U.K.), in U.S.A., where many of the early findings were made, and in Canada, U.S.S.R., Africa, South America, India and Japan. As soil analyses slowly extend in the under-developed countries, the same types of soil deficiencies or excesses, and the same animal diseases associated with them are being increasingly diagnosed. Paucity of analytical staff, of laboratory facilities and of trained agricultural advisers makes progress slow. There can, however, be no doubt at all that from scores of millions of hectares of the less-developed and hungry world the local production of human food could be greatly increased by the application of micro-nutrients, in appropriate quantities, to deficient soils.

Soil 'deficiency' in micro-nutrients, in the sense used here, requires consideration. Even a 'deficient' soil may contain, in the levels penetrated by plant roots, most of the essential trace elements in quantities several times greater than the amounts removed per annum by any crop. Thus in investigations of oat yields in relation to soil copper content (Kannenberg, 1966; Perkow, 1966) on humus-containing sandy soils in Western Europe, a total soil Cu content of 4 ppm appeared to be minimal for healthy growth of this cereal, but the amount taken up by one crop was only about 0·4% of the *total* Cu. But some of the soil Cu (and this applies to other micro-nutrients as well), appears to be unavailable to the plant, so that the proportion of the *assimilable* Cu taken up would be substantially greater than 0·4%.

It was found that where, in such soils, the total Cu amounted to only 2–3 ppm deficiency symptoms occurred, and Cu dressings sufficient to double this figure were applied. A fine powder, made from waste copper metal, or from brass scraps from the metal-working industries, provided a useful, long-acting corrective, when distributed over the deficient area, for the original shortage (Kannenberg, 1966). Even finely-ground slag from copper extraction or refining processes which may have a Cu content as low as 0·5% will, if added in powder form in sufficient quantity, meet the copper needs of the soil and plant, beside providing a (somewhat uncertain) supply of other micro-nutrients (and possibly some toxic substances). The beneficial action of the soil dressings just mentioned is said to be more persistent than the effect of an equal quantity of Cu in the form of the more readily leachable copper sulphate. Whichever method is used, the effect of the annual removal, by crop after crop, of small but appreciable amounts of Cu, Mn, Zn, B, etc., from the soil, together with the often more serious loss of these elements by leaching may eventually reduce, in intensively cultivated areas, the soil content of at least one of the trace elements to below the sufficiency level unless appropriate dressings are repeated at intervals or the ordinary fertilizers or other manures are sufficiently 'impure' to re-imburse the soil losses.

In several parts of the U.K. the Agricultural Advisory Services have, in recent years, been paying increasing attention to variations, either above or below the normal range, in the micro-nutrient (as well, of course, as the macro-nutrient) status of soils, and to the effect of these variations on farm crops and on grazing animals. Thus in East Anglia (Patterson, 1965) serious shortages of available soil Cu and/or Mn have been found, extending over large areas—at least 12,000 ha (*ca.* 30,000 acres) of mainly, but not exclusively, peat soils. A dressing of crude copper sulphate at 14 kg of Cu per hectare is needed for some of the deficient land and is effective for up to 8 years;* any manganese deficiency must be corrected at the same time if the crops are to benefit fully. Yields of cereal crops have been improved by the substantial average of some 1200 kg/ha; carrots and sugar beet have also shown marked responses.† It would appear that animal health in these districts has benefited. In some areas, foliar sprays on the growing crops—using much smaller amounts per hectare of the trace element, but where a subsequent crop will show little benefit—have been used with some success. Since Cu^{++} is a very effective molluscicide, it would be interesting to know whether part of the beneficial effect of copper applied either direct to the soil or as a foliar spray is to reduce the destructive slug or snail population. It has been stated (Macfadyen, 1964) that a large proportion of the primary production of grassland that is eaten by herbivores is eaten by animals other than grazing cattle.

From the standpoint of ruminant nutrition and husbandry, soil and fodder analyses for micro-nutrients have a special importance. This is because a diagnosis, from the physical condition of the animal or from a diminution in production alone, that it is suffering from a sub-acute dystrophy that is ascribable either to a moderate deficiency or a somewhat toxic excess of this or that micro-nutrient, is a matter of great difficulty. Lack of 'thriftiness', loss of condition (a very subjective assessment), slow growth, low fertility, anaemia, diarrhoea, a drop in milk yield, all may be due to one or many causes —infection, ingestion of a toxic plant or even a piece of wire in the rumen. Direct analyses on blood, or milk in the lactating animal, or on other animal tissues obtained by biopsy have been increasingly used in very recent years. In the case of suspected copper deficiency, blood and liver analyses, including the assay of the copper enzyme cytochrome oxidase in the liver, have given useful leads, as has the level of liver cobalt, or of vitamin B_{12} in blood or liver (or in milk in the case of a lactating animal), in suspected Co deficiency. The level of zinc in the plasma is a fair indication of the status of an animal in respect of that micro-nutrient. But in most of these analyses, the extent of the range of values for a normal, healthy, adequately fed animal is still a matter of uncertainty.

The problem of *preventing* nutritional disease in grazing stock in regions where the soils or plants are short of available micro-nutrients is easier of solution than the one

* It is useful to remember that kilograms per hectare are approximately equal to pounds per acre.

† It has been estimated that the improvement in return, to the farming community of East Anglia for correcting Cu deficiencies, is not less than £250,000 per annum.

which arises in areas where these substances are present in toxic quantities. In the former case, deficiencies *when diagnosed* may be made good by suitable soil dressings, by mineral licks or by direct administration of the missing trace element to the animal; in the latter case the area may be quite useless as pasturage or for producing edible crops. Fortunately, such toxic soils are uncommon, but there are larger areas with less marked toxicity where stock can be pastured for limited periods only, after which a transfer elsewhere—often a costly nuisance to the farmer—must follow.

Areas have been known for many years in the U.K. and elsewhere where Cu, Mo or Se occur naturally (i.e. apart from mining areas or spoil dumps, and away from the vicinity of smelting or refining works) in amounts sufficient to poison grazing farm stock. A classic example of this is the 'teart' areas of Somerset, said to cover some 10,000 ha. If pastured on teart land, ruminant animals, particularly the cow in milk (whose intake of food may be more than double that of the same animal when not lactating), and also young bovine stock, formerly suffered scouring of different degress of severity. This could end fatally if the animals remained long on such pastures. Even moderately teart pastures reduced 'thriftiness' and fertility. It was found (Ferguson, Lewis & Watson, 1936; Lewis, 1943) that the cause was a high molybdenum content of the soil and the herbage—the latter could contain as much as 150 ppm of Mo calculated on the dry weight. Advantage was taken, later, of the empirical finding that there was an 'antagonism' in the ruminant between the two micro-nutrients Mo and Cu (an antagonism also found in some non-ruminants including the rat and the rabbit). When the scouring cows were treated with substantial daily doses of 1–2 g of copper sulphate admixed (and often cubed) with much larger quantities of normal feed supplement the diarrhoea and other symptoms disappeared. An animal so treated could, if the treatment were maintained, continue to graze teart pasture with impunity. Horses, it was known, could graze such pasture unharmed, without the Cu protection.

Molybdenum in moderate amounts in the diet of cattle—say of the order of 10–20 ppm is not far removed from having a mildly toxic action, but it is almost certain that such amounts have the more important effect of bringing about a virtual copper deficiency in these animals, which have a fairly high copper requirement. Sheep are more resistant to the effects of additional dietary Mo; horses still more resistant. In sheep the addition of Mo to the diet induces the same type of steely wool as occurs on a Cu-deficient diet (Dick, 1954). The direct experimental finding from the same Australian laboratory, that the Cu content of the livers of ruminants on a diet high in Mo is diminished, lends further support to the view that a major part of the effect of an increased intake of this trace element is to induce a copper deficiency. If there is sufficient Cu in the diet to approach toxicity—such as may occur when in Australia sheep are grazed on pastures with a high proportion of subterranean clover (which has a high Cu content)—extra Mo in the diet or in salt licks affords protection.

The Mo–Cu relationship is a complex one; the dietary intake of sulphate and of protein plays a part. In the non-ruminant, if the amount of sulphate is high, the

deleterious effects of high dietary Mo are reduced or eliminated; a similar effect of high protein is probably due to the presence in the protein of amino acids containing sulphur which is convertible, in the organism, into sulphate. In either case Mo-induced copper deficiency is to a greater or lesser extent alleviated. In the ruminant the position is different (Dick, 1956; Mills, 1964); it would seem that sulphate enhances the action of Mo in inducing a copper deficiency. There is a considerable body of evidence from different sources on the Mo–Cu–sulphate–protein relationship but as yet it does not fall smoothly into line. The reasons for this doubtless lie with variables such as the amount of other micro-nutrients, or of major ones such as calcium, or of naturally occurring chelating agents. One of these, phytic acid, if present in sufficient quantity, is known to reduce the availability of several trace elements, including Cu itself and Zn.

A few of the individual micro-nutrients, and fairly recent findings regarding them, will be discussed in the following paragraphs.

Copper

Excluding iron, which as far as mammals are concerned should be regarded as distinctly more than a micro-nutrient, more experimental work has been done and more known about the part played by copper than about that of any other of the essential trace elements needed by this class of animals. Much information has been gained in the last 20 years about its role as an irreplaceable constituent of a number of important enzymes and of its occurrence in several other protein complexes that do not appear to act as enzymes.

It is of interest briefly to consider copper in the human body from the quantitative standpoint. It must be present in all the 600×10^9 cells of the adult body but the total quantity present in an adult man is no more than 100–150 mg. There is a daily metabolic loss which averages 2–3 mg only, an amount easily replaceable from a normal mixed diet. The total amount of copper in the body is intermediate between that of iron, which is about thirty times greater, and that of iodine which is some six to eight times smaller (though the function of the last as an essential trace element has been recognized far longer than that of copper).

It perhaps does not lead one very far to estimate the total quantity of this or that element in the body, except that it does give a useful idea of what 'trace' means in relation to the macro constituents to realize that the ratio Cu : N in the adult human body is 0·05 : 100. On the other hand, in the copper enzymes the order of the relation Cu : N is nearer to 3 or 4 : 100.

The different organs of a human adult on a normal diet vary in their copper content by at least a factor of ten. Of the major tissues the figure for liver is the highest at about 25–35 ppm on the dry weight (Cunningham, 1931; Bruckman & Zondek, 1940). (This figure does not necessarily apply to other mammals, particularly to mature ruminants.)

Of the small quantity of copper present in the food eaten by the normal human adult, only a few per cent, probably not more than 10%, is actually absorbed. The quantity of copper lost in the excreta varies from day to day but over a period of, say, a month, it is approximately equal to the amount absorbed; an exception occurs in pregnancy when there is a positive Cu balance and also in Wilson's disease, to be mentioned later. Of the Cu 'turned over', a minute proportion reaches the urine, but almost all is excreted via the bile. Whilst a small part of this may be reabsorbed, most of it goes into the faeces [findings based on the use of radioactive ^{64}Cu (Walshe, 1966)]. ^{64}Cu administered to man in the diet appears, after absorption from the intestinal contents, as a loose complex with serum albumin. From this complex it is picked up by the liver and leaves that organ to enter the circulating blood as 'ceruloplasmin', a protein with enzymic properties.

Any mixed human diet containing sufficient protein (say 1 g/kg body weight) especially animal protein, or enough iron, will contain sufficient copper for the normal adult and there is general agreement that copper deficiency symptoms in the human adult, i.e. specifically curable by Cu therapy, have never been demonstrated. A diet containing, from time to time, liver or kidney or meat or leafy vegetables will assure an ample supply of copper. A regime of oysters or other shell fish (which may contain up to 0·03–0·04% Cu), though hardly to be recommended as an exclusive diet, would deluge the digestive system with copper. But increasing the intake does not greatly increase the amount of copper absorbed.

The infant, if kept entirely dependent on human or bovine milk may suffer a lowering of the level of copper in the blood, though without any overt symptoms of copper deficiency. Most mammals at birth, including the baby have a reserve of copper (as of iron) in the liver, possibly associated with the high ceruloplasmin content found in the maternal blood during part of her period of pregnancy. Milk is one of the few foods high in protein [about 17% (on the dry weight) for human milk and about 26% for cow's milk] that contain little copper (or iron), but the normal human infant, on a milk diet, with the help of its liver reserve, of the small copper supplement derived from its less hygienic encounters with its surroundings and in view of its relatively slow rate of growth, seems to be just about able to meet its immediate copper requirements.

The very young piglet, like the human baby, possesses a fairly substantial reserve of copper in its liver at birth—a figure of the order of 200 ppm of dry liver tissue seems for this species to be normal at this stage. It doubles its birth weight in 10 days, as compared with the 180 days or so that the human infant requires. Its demands for both copper and iron are sufficiently far above what unsupplemented sow's milk can provide for it to suffer invariably from lack of iron, and usually from lack of copper also, if it is kept in a concrete sty, away from soil and grass, and with little access to its dam's food. It is rather surprising that, in the course of evolution, the composition of sow's milk adjusted, as it is, in its high content of protein, fat and 'mineral' constituents such as Ca, P and K, to the large requirements of the rapidly growing suckling, is not also

adjusted to the latter's large Fe and smaller Cu needs. The answer is, perhaps, that this animal did not evolve in a concrete sty. One might add that there is some slight tendency in the sow, as in all the other species examined, to provide in the colostrum and early milk a somewhat higher concentration of Fe, Cu and some of the other trace elements, than in later milk. It is also true that sow's later milk contains nearly twice as much Fe (some 1·8–2 ppm) as human milk or cow's milk, but the amount in any case is insufficient* for the growing piglet unless it has—as it would have under natural conditions—some other source of iron.

Milks of other species show similar characteristics, in that they are adjusted, in their concentration of protein, fat and minerals to the demands for tissue and bone formation suited to the growth rate of the young of the particular species, but are inadequate as regards Fe and Cu. Addition to the dam's feed of several times her normal dietary intake of Fe or Cu has practically no effect on the level of these in her milk. This is not the case with most of the other micro-nutrients, since the concentration in milk of Mo, Mn, Zn, Co, Se and I can be markedly increased by the addition of a suitable salt of any of these trace elements to the diet of the lactating animal. The mammary barrier to transfer of the trace elements to milk seems a little erratic; the reason for this would probably be worth investigating.

One of the results of copper shortage in the diet of lactating cows (and this doubtless applies to other species as well) is to decrease the yield of milk. This has been reported as affecting a large number of cows in the Netherlands, if their pasturage is even moderately Cu-deficient. With more severe Cu shortage there is not infrequently de-pigmentation of the hair.

The marasmus (wasting) syndrome is well known in tropical, hungry countries as afflicting many young, recently-weaned children when mothers' milk is replaced by an inadequate carbohydrate diet, short of both protein and calories. This syndrome, which is generally accepted as being the result of such a diet, may be conditioned in part by the fact that the previous diet, almost exclusively milk, of these children came from mothers also suffering from defective diets. Dietary shortages of any of the micro-nutrients that have been investigated, such as copper, lower, as might be expected, the concentration of that micro-nutrient in milk and usually lower milk yield as well. The mothers of these children are, usually, themselves short of protein, with which, it will be remembered, the micro-nutrient elements are very frequently associated. Further, late lactation milk of all the species examined contains considerably less copper than even the small quantity present in early milk (in normal woman's milk, about 0·6–0·9 ppm in early milk falls to 0·15–0·17 ppm in late milk) (Underwood, 1962, p. 66). It is well known that both in marasmus and kwashiorkor there is almost invariably a

* Very occasionally a sow is found which can apparently maintain her offspring on milk alone without their suffering from anaemia, but a thorough investigation of such an animal does not appear to have been made. There may possibly be a small leakage of red cells into the milk in such a case.

greatly diminished intake of iron, vitamin A and other vitamins, and of sodium, potassium and magnesium. There can be little doubt that in many cases there is also a shortage of copper. Determinations of copper in dry, fat-free liver tissue show that in kwashiorkor it contains very significantly less Cu than control, normal tissue; the hair, too, is significantly lacking in Cu (McDonald & Warren, 1961). Infants recovering on a milk diet from severe marasmus improve more rapidly when the diet is supplemented daily with 2·5 mg Cu, given as copper sulphate (Graham, Cordano & Baertl, 1963).

The copper enzymes are, without exception, concerned with systems in which oxidation or electron transfer takes place as an integral part of a series of enzyme-catalysed reactions, but why and how *copper* plays an essential part in their catalytic function is still uncertain. To quote an American authority (Mason, 1966) 'copper proteins have a wide range of function for structural reasons that are not understood' and again 'no single ligand of copper in any copper enzyme has yet been identified'. Nevertheless, much research effort has, during the last few years, been applied to the solution of these problems, as also to the somewhat analogous questions concerning other enzymes which contain a trace metal (e.g. Mo, Mn or Zn) as an essential structural constituent. [A good deal of the recent work on these problems in relation to copper is to be found in *Biochemistry of Copper* (Mason, 1966).] A brief mention of a few of the known copper enzymes is not out of place.

Cytochrome C oxidase, which contains in its molecule haem as well as copper, is a very complex and, at least in mammals, a ubiquitous enzyme—it is present in the mitochondria of all tissues and is closely concerned with tissue respiration. When separated from animal (bovine) tissue, the purified enzyme appears to contain a molecule of iron for each molecule of copper. Iron is also associated with some of the other copper enzymes either in the enzyme structure (as in cytochrome oxidase) or in close juxtaposition in the sub-cellular organelle (e.g. the chloroplast) in which the copper enzyme functions. Deficiency of cytochrome oxidase in liver tissue has already been mentioned as a clear indication of a dietary shortage of copper or of a defect in copper assimilation from the diet (C. F. Mills, private communication). This deficiency occurs in liver, kidney or brain tissue, even before the anaemia, resulting from copper shortage, becomes marked. A cytochrome C oxidase with a different composition from the animal one, but still containing copper, is present in some micro-organisms.

Another copper enzyme widely distributed in plants and bacteria is ascorbic acid oxidase (or ascorbate oxidase). It is a complex protein with a molecular weight of about 140,000 and appears to contain eight Cu atoms in the molecule.

Tyrosinase, a polyphenol oxidase of low specificity but, as regards its date of discovery, of considerable seniority, is also widely distributed, in bacteria, fungi, plants and some animals, and appears to contain two different types of active centre, each containing copper (Dressler & Dawson, 1960). Amine oxidases, each containing copper, have been shown to occur in several tissues, including the mitochondria of bovine and other mammalian livers; their activity seems to be proportional to the amount of Cu in

the different preparations. The enzyme from mitochondria appears to be a flavoprotein (Hawkins & Bjur, 1952).

Plastocyanin, with enzyme properties akin to those of ascorbate oxidase occurs in the chloroplast and plays a part in the chain of reactions occurring during photosynthesis.

In the normal human adult, the 0·12±0·015 mg/100 ml of Cu in blood is divided almost equally between the red cells and the plasma, but the copper inside the cell is combined differently from that in the plasma. Within the red cell some 60% of the copper is present as erythrocuprein, a protein which has been crystallized and contains some 0·38% of Cu—two atoms both Cu^{++}—and has a molecular weight of 33,600 (Stansell & Deutsch, 1965). It is possible that a similar cerebrocuprein from brain and a hepatocuprein from liver, both from human tissues, may be identical with the erythroprotein from human erythrocytes (Porter, Sweeney & Porter, 1964). All have similar copper content, amino acid composition, molecular weight, absorption spectra and other physical characteristics.

Of the copper circulating in human blood plasma (and in that of other species including avian species) 80% or more of the copper is in the form of the blue protein ceruloplasmin, an oxidase with, at least *in vitro*, a varied assortment of substrates. Its large molecule, like that of ascorbate oxidase from plants, contains eight atoms of copper, some of them in the cuprous state. The crystalline protein, which contains 0·30% of copper has a molecular weight of about 160,000. Its amino acid composition (Kasper & Deutsch, 1963) has been investigated and it also contains about 8% of carbohydrate. It may possibly consist of eight sub-units. For full enzymic activity it requires ferrous iron as a co-enzyme. In buffer solution it will actively oxidize ascorbate at pH 6–7·5 but will not do this in blood plasma which contains inhibitory substances. A low plasma level of ceruloplasmin is usually indicative of a deficiency of copper in the diet, but this protein is also low in Wilson's disease in man, where a dietary shortage is *not* the cause, and in nephrosis.

Before the plasma level of ceruloplasmin in a healthy individual can be raised above the usual level the diet has to contain a large excess of copper, but in pregnancy (on a normal diet) quite high levels, about twice the non-pregnant level, are often found. The physiological role of ceruloplasmin is still uncertain, but studies during the last few years of Wilson's disease, a somewhat rare, genetically determined error of metabolism in which copper accumulates in the tissues, have thrown some light on the normal metabolism of this micro-nutrient and the possible part played by this copper protein.

Sufferers from Wilson's disease have, on a normal diet, a positive Cu balance, and, unless treated, die early, usually in childhood or early adolescence, as a result of the toxic condition resulting from the eventual overloading of the brain and liver tissues with copper. In this disease serum ceruloplasmin is always low, i.e. of the order of 25% or even less, of the normal but what there is of it appears to be the normal protein. Using radioactive ^{64}Cu, it has been found that transport of copper in the body is

abnormal and the radioactive metal is hardly detectable in ceruloplasmin obtained subsequent to its administration.

On the basis of a volume of experimental evidence it has been suggested that an enzyme complex in the liver, which, as mentioned earlier, in normal individuals picks up the copper from its loose combination with serum albumin (a combination formed when dietary Cu is absorbed from the intestine) and transforms it into ceruloplasmin which is then liberated into the blood plasma, is absent or defective in Wilson's disease (Osborne & Walshe, 1964). Consequently absorbed copper finds its way in abnormal amounts into various tissues. As the disease progresses, the steadily accumulating copper in the brain slowly poisons the membrane adenosine triphosphatase; in the liver it causes cirrhosis and in the kidney severe damage to the tubules. Attempts have been made in recent years to prevent the hitherto inexorable progress of the disease by treating the patients with copper-binding, chelating agents such as BAL (dimercapto-propanol) and with *d*-penicillamine (2,2-dimethylcysteine) (Sherlock, 1961), the latter in the rather heroic doses of up to 3 g daily, given by mouth. These attempts have met with some success, particularly with penicillamine, which has markedly increased the urinary excretion of Cu, and has relieved both the nervous and hepatic symptoms in a substantial proportion of the sufferers.

Though the *in vivo* function of the blood ceruloplasmin is still uncertain, it may well be that in normal individuals it forms, in association with the liver, an essential part of a homeostatic mechanism which assures a sufficient but not too high level of copper in the tissues and thus maintains, despite fluctuations in the day-to-day intake of this element in the diet, a steady level of the intracellular copper enzymes.

Like most of the essential micro-nutrients, copper can be toxic to normal mammals if given in sufficient quantity; if it is given by mouth the amount needed to produce acute symptoms is nevertheless several scores if not hundreds of times greater than the average daily intake from a normal diet. Wilson's disease shows that the accumulation, over a period of years, of this element in human tissues becomes lethally toxic when concentrations of the order of 100–200 ppm (on the dry weight) in the brain, and up to 800 ppm (on the dry weight) in the liver are attained. In pigs, however, considerably higher figures for liver copper occur after feeding for some weeks to weanling animals rations containing 250 ppm of Cu, and that without deleterious effects; indeed with beneficial results both on the growth rate (an average increase of 11% on the daily weight gain) and on feed utilization (as average improvement of 7%). Literally millions of growing pigs in the U.K. and elsewhere are now being given diets containing 0·1% of $CuSO_4.5H_2O$, or its equivalent as cupric oxide (Braude, 1966). If the amount of copper is increased much above this, the pigs begin to reject their rations, and if compelled to eat diets containing the enormous quantity of 750 ppm of copper, poisoning in the shape of anaemia, liver damage and jaundice results (Annual Report, Rowett Research Institute, 1964, p. 42, Aberdeen). Addition of 500 ppm of zinc to the high Cu ration reduces tissue damage and eliminates the jaundice but not the anaemia. A

supplement to the high Cu ration of 750 ppm of iron protects against tissue damage and jaundice and prevents the rapid decline in haemoglobin in the blood. The actual requirement of the pig for Cu for 'normal' growth is of the order of 250 times *less* than the amount in the growth-stimulating ration containing 250 ppm.

This high mammalian tolerance for copper is the reverse of the acute sensitivity shown by many molluscs and by most species of parasitic fungi. The snails which harbour the bilharzia parasite at one stage of the latter's life cycle are highly intolerant to copper. One part of Cu (or less) in a million of pure water is lethal to these molluscs. In a stream, irrigation channel or pond, where some of the Cu added as a molluscicide is picked up by impurities and by other small creatures, a larger concentration of Cu is needed. A surprising toxicity shows itself in certain circumstances in the pigeon, where fatal convulsions usually follow the injection of as little as 10 μg of Cu^{++} into the sub-arachnoid space. The copper interferes with a $-SH$ group of the ATP-ase in the microsome membranes in the brain tissue and inhibits this enzyme, with a consequent breakdown in the transport of ions across the membrane and violent convulsions in the bird (Peters, Shorthouse & Walshe, 1965).

Copper in food technology

The catalytic action of traces of copper in accelerating oxidative changes, most of which have a damaging effect on the flavour in many foodstuffs, is well known. Milk, for example, with an original Cu content of some 0·2–0·3 ppm, picks up copper surprisingly rapidly from a naked copper surface. In making Emmenthal cheese, however, a copper vessel seems to have advantages over a stainless steel one. About 1 ppm Cu is take up within ½ hr of placing the milk in such a vessel, and some 1·6 ppm within the next 10 hr The temperature of the milk appears to have little influence. The additional copper is advantageous for the normal development of the propionic and lactic acid bacteria and for the formation of the characteristic eyes in this type of cheese (P. Kästli, private communication). Too much copper is deleterious.

Flavour in milk is highly sensitive to the presence of additional copper, and for the maintenance of satisfactory organoleptic quality in milk to be used for human consumption, either after pasteurizing or drying, it is most undesirable for it to have been in contact with a copper surface.

Selenium

It has been known for many years that selenium, in fairly large amounts from the trace element standpoint, is toxic to most animals, including man, but it is only about 10 years ago that its status as an essential micro-nutrient first became probable, when 'factor 3' present in some samples of brewers' yeast, which prevented liver necrosis in rats on a restricted diet deficient in vitamin E, was shown to contain, as the active constituent, a selenium compound (Schwarz & Foltz, 1957). Chickens also, on similarly

restricted diets developed a lethal 'exudative diathesis'. Both these dystrophies were prevented when either 'factor 3' or a small quantity of (selenium in the form of selenite) or vitamin E was added to the diet. More recently it has been shown that, even in presence of vitamin E, diets deficient in selenium will not support normal growth either in young rats or chickens on experimental diets, but that the addition of a minute amount of selenium—of the order of 1 ppm or less of the diet—will restore the growth to normal.

The position of Se as an essential trace element for farm animals under field conditions was established by findings in Scotland (Blaxter & Sharman, 1953) and elsewhere. These resulted from a study of the disease in cattle and sheep known as 'white muscle disease' or nutritional muscular dystrophy. Of the beef calves from a muscular dystrophy area in north-east Scotland, 20% or more of the animals in any one herd could be affected, but selenium at the rate of about 0·5 ppm of the weight of the calf, given as sodium selenite, either by injection or by mouth and administered either in one dose at birth or in a number of smaller doses during the first few weeks of life, provided an adequate preventative (Sharman, Blaxter & Wilson, 1959). Administration of large amounts of vitamin E as α-tocopherol acetate was also found to give some protection to calves, though it was not as effective as selenium. Lambs in such areas could be protected either by direct administration of Se to the animals after birth, or by giving to the ewe one dose of 5 mg of Se as sodium selenite—equal to about 0·15 ppm of the weight of the ewe—3 weeks before lambing. Se seems to cross the placental and also the mammary barrier without difficulty. The level in blood serum of the enzyme glutamic–oxalacetic transaminase is significantly increased in progressive muscular dystrophy in human subjects. In threatened and actual muscular dystrophy in calves or lambs the same occurs and provides a diagnostic test. The amount of enzyme per unit volume of serum is reduced by the injection of either selenium or α-tocopherol acetate.

Once attention had been called to the very beneficial effect of minute quantities of selenium on animals suffering from muscular dystrophy as a result of a selenium-deficient diet, further well-controlled experiments with farm stock indicated other merits of this micro-nutrient. These included the improvement of fertility in ewes (Scotland), the prevention of periodontal disease in sheep (New Zealand), of prenatal myopathy in lambs (U.S.A.) and both reduced mortality and increased growth-rate in lambs (New Zealand). Increases in fleece weight in sheep have been reported from Canada and recently confirmed (Quarterman, Mills & Dalgarno, 1966) by findings in Scotland. In an experiment in a selenium-poor district in New Zealand (Hartley, 1961) where many grazing areas have been shown to have soils deficient in this element, it has been found that 10 mg/month of Se, given by mouth, markedly raised the growth rate of beef calves. It is stated that their weight increased above that of the untreated control animals by as much as 33% in 11 months. It is, of course, to be expected that the most striking results of the administration of any essential micro-nutrient to grazing farm stock will be obtained when soils or pastures are grossly deficient in the particular trace

element. Fortunately most soils and pastures in the U.K. contain enough selenium to prevent at any rate *acute* deficiency symptoms in grazing stock, though in places where such animals are found to be 'bad doers', an investigation for possible trace-element deficiencies in the soil should not rule out selenium. The effects of acute deficiency of selenium (or of selenium and α-tocopherol) are not confined to grazing stock. On a diet short of vitamin E, of sulphur amino acids and of selenium, some strains of rats, in the course of 1–3 months, develop liver necrosis, preventable by the addition of 0·5 ppm or less of selenium to the diet (Bunyan, Green & Diplock, 1963).

No mixed diet for human consumption is likely to be unduly low in selenium, and no occurrence of selenium deficiency in the adult appears to have been recorded. It has however been reported that there is a type of kwashiorkor in young children that can be relieved by a daily dietary supplement of 25 μg of Se (Schwarz, 1961). Unlike Cu, Mo or Zn, selenium does not seem to form an integral part of any tissue enzyme molecule, though it may possibly stimulate the activity of certain enzymes (Massey, 1953).

Like several of the other essential trace elements, selenium is toxic to animals, including man, if administered, or taken in the food, in quantities still small but several times larger than those required for normal growth and health. As regards the factor of safety, it is rather more toxic to mammals than cobalt, copper or manganese. The so-called alkali disease, a chronic disorder of horses and cattle, has been known in Nebraska and other parts of the Great Plains of U.S.A. for more than a century—long before selenium was suspected of having an effect on animal health. Since then, the disease has been observed in many other countries, as far afield as Canada, Ireland, Mexico, Colombia, Australia and the U.S.S.R. In its more acute and lethal form, 'blind staggers', it has been met with in several western States. It was not till 1933 that the presence of Se in the soil in more than very small amounts (say a total soil Se of more than about 0·5 ppm) and in plants growing on such seleniferous soils, was established as the cause of this disease. It was later found that the irregular occurrence of 'alkali disease' on soils containing abnormal amounts of Se was due in part to the fact that the soil Se could be present in several different states of combination, not all of which were equally available to the plants, and in part to the markedly different ability of different pasture or range plants to absorb Se from the soil. Some took up only small amounts of this element from the soil; others not only absorbed it freely, but concentrated it in their leaves and other tissues, with disastrous effects on the grazing animal consuming them.

In general, ordinary grasses, which do not grow well on the more highly seleniferous soils, take up relatively little Se (about 1–5 ppm on the dry weight), cereals will usually take up more (the grains may contain 30 ppm or more) and couch grass may contain over 200 ppm. *Astragalus* (milk vetch), *Neptunia* (also a legume) and *Haplopappus* (a composite) are even more active concentrators of Se and may contain thousands of ppm. These three kinds of plant *prefer* to grow on seleniferous soils, which most other species do not. They are thus useful indicators of the presence of such soils. It is stated

that *Astragalus racemosus* and certain other indicator plants grown on seleniferous soil may contain sufficient Se to give them a nauseating odour, presumably resulting from a volatile Se compound. (Anyone who has had the misfortune to work with Se compounds will be only too well acquainted with the foul smell of some of them, particularly of H_2Se.)

Though these very large quantities of Se in the accumulator plants appear to have no harmful effect on the plants themselves, they can be very harmful indeed to animals eating them. Since the margin between the beneficial and the toxic effects of Se in the animal dietary is small—0·1 ppm of Se in the diet is sufficient to prevent Se deficiency symptoms whilst 1 ppm which could be readily obtained from accumulator plants grown on Se-dressed soils could very easily be toxic—the addition of this trace element to a Se-deficient soil is a somewhat dangerous procedure. It is said to be wiser to administer the Se direct, either subcutaneously as Na_2SeO_3 or intramuscularly in oil as $BaSeO_4$, to such animals as are otherwise likely to suffer from Se deficiency.

Accumulation of, and tolerance for, trace elements

The phenomenon, fairly widespread in both animals and plants (of which certain plants growing on seleniferous soils provide an excellent example) of the apparently haphazard ability of certain organisms to accumulate, with apparent impunity, 'trace' elements with no obvious metabolic functions, in amounts toxic to other animals or plants, is not easy to account for. It is not quite comparable with the synthesis by protista, plants or animals of compounds such as poisons or antibiotics, the functions of which (but not, unfortunately, the chemical mechanism by which these usually complicated substances have been evolved) seem fairly clear. A few examples may be quoted, such as the accumulation of barium in the fruits of *Bertholletia excelsa* (Brazil nut), of bromine in sponges, molluscs and corals, of copper in the hepato-pancreas of the wood-louse (Weiser, 1961), of cobalt in *Clethra barbinervis*, of fluorine in the leaves and seeds of some species of *Dichapetalum* (Peters, 1961) of manganese in certain sponges, of nickel in *Alyssum bertolini* (a crucifer), of niobium, tantalum and vanadium in some ascidians, of zinc in *Thlaspi alpestre* (a crucifer), and several other plants (Vinogradov, 1959; see also Bowen, 1966a). A large proportion of the now fairly numerous occurrences of such accumulation reported in the literature is of elements which, in small amounts, are essential micro-nutrients.

In some of the cases quoted, as in those of the enormous accumulation of Se, Ni or Zn in certain tolerant plants, the obvious explanation is that in the course of ages these organisms have, in the evolutionary struggle for survival, found a habitat in which rivals could not live, but why should they accumulate such very large amounts of the toxic element in their tissues? Is it that a second advantage is thereby conferred—the destruction of predators? The accumulation by certain ascidians of rather rare metals

such as vanadium, niobium or tantalum from sea water containing vanishingly small concentrations of these elements seems to be equally difficult to explain.

As regards selenium, it has been found that the proteins of plants growing happily on seleniferous soil contain analogues, such as selenocystine and selenomethionine, of the sulphur amino acids.

Whilst selenium is highly toxic to most insects and mites and, as a pesticide, is in fairly wide use in horticulture in the form of sodium selenate, some insects are remarkably immune to it. The larvae of certain beetles and chalcids can survive and complete their life cycle on foods like the seeds of the poisonous *Astragalus* sp., seeds which contain about seventy times as much Se as is needed to kill mammals and most other insects. Species of adult beetles, flies and grasshoppers have also been observed to feed on these plants and to contain in their tissues, without showing any injurious effects large quantities, of the order of 10–20 ppm of Se (Moxon, 1939; Fox, 1943; Moxon & Rhian, 1943).

Somewhat analogous to the action of copper in preventing Mo poisoning in stock grazing on 'teart' land, arsenic was found, in work published several years ago, to protect rats against the toxic effects of dietary Se (Moxon, 1938). Later, farm stock, including cattle and pigs, ingesting toxic amounts of selenium have been protected by the addition of sodium arsenate to their diet; the amount of arsenic being insufficient to be toxic by itself. There are obvious difficulties in applying this kind of anti-Se therapy in practice on the farm but forms of As less toxic than arsenate or arsenite have been used with some success. Increasing the protein content of the ration, or the addition to the diet of fairly substantial amounts of sulphate (Halverson & Monty, 1960) are methods which have been used to provide some protection of animals against Se.

There is some, not very clear-cut, evidence that symptoms of Se poisoning may occur in humans in seleniferous areas where the rural consumers subsist mainly or exclusively on home-grown products. It is stated that in such areas gastro-intestinal disturbances, mild jaundice, dental caries, inflammation of the gums, dermatitis, damage to finger- and toe-nails and loss of hair occur to a greater extent than in similar human populations in non-seleniferous areas. A little more definite is a human syndrome, occurring in Colombia in areas with a high soil Se, in which hair and nails are lost, and which appears to be the result of selenosis. Animals in these regions show clear symptoms of Se poisoning.

Fluorine

The question as to whether small, carefully controlled quantities of fluorine should be added to drinking water supplies deficient in this element has been somewhat emotionally debated in Britain in recent years. A few fairly recent findings, obtained either experimentally or by careful survey, may be usefully included in the present article.

The occurrence of small amounts of combined fluorine in most plant and animal tissues has been known for over 150 years, since the analytical work of Berzelius, Klaproth and others. They showed that the bones and teeth contained very considerably

more F than the other tissues examined. It has since been found that the amount in normal human bone steadily increases with age.

It has also been known for some 35 years that in regions where fluorine is present in the food or drinking water in appreciably greater quantities than normal, both the animal and the human inhabitants frequently show tooth defects ('mottled enamel') roughly parallel in their severity with the amount of fluoride in the water (Ockerse, 1941). During the investigation of these tooth defects it was also noticed that in high fluorine areas the occurrence in the human population of dental caries in the young, or of loss of teeth owing to previous dental caries in the older members of the community, was much less frequent than in those areas where the fluorine in the drinking water was minimal.

Later it was established that the incidence of dental caries in children examined at 12–14 years of age was greatest amongst those who had been living in areas where the fluorine content of the drinking water was least, and that there was a direct quantitative relationship between these two parameters (Dean, Arnold & Elvore, 1942). The margin, if any, between the fluorine content of the water that was effective against caries and the content which began to produce unsightly mottling of the teeth became a question of obvious importance. A careful study, conducted in twenty-one cities in U.S. with widely varying amounts of fluorine in their drinking water, showed clearly that if the water contained between 1·0 and 1·5 ppm of fluorine (calculated as F) dental caries was less by about 65% than with water containing well below 1·0 ppm whereas 'mottling' began to be observed only at about 2–3 ppm of F (Dean *et al.*, 1942).

On the basis of these findings a 10-year programme was arranged in three U.S. and one Canadian town, in each of which the municipal water supply was very low in fluoride. In each town, sodium fluoride was added to the water to raise the F content to 1 ppm. Four nearby, untreated towns, were used as controls. The results of this work, published in 1956, clearly demonstrated that the fluoridization of the water had resulted, in each town, in a diminution in the incidence of dental caries in the resident children (6–10 years of age) of approximately 60%, without any deleterious effects (Arnold *et al.*, 1956; Brown *et al.*, 1956).

Findings from carefully controlled field experiments in several areas of New Zealand, Holland and the U.K. have confirmed the American results. Between 1·0 and 1·2 ppm of F in the water supply reduces greatly the prevalence of caries both in the decidous and the permanent teeth of children resident in these areas in the vulnerable period up to 10 or 12 years of age, without causing mottling (Bransby, Forrest & Mansbridge, 1963). The evidence for the value of fluoridization in preventing a major part of the distress caused in children by dental caries is now massive.* It is stated

* A. J. Dalyell-Ward (1963) provides a noteworthy sociological study of the astonishing, organized opposition to fluoridization and outlines a public relations programme needed to effect the education of all concerned.

that in 1963, as a consequence of these very clear-cut findings, over 44 million people in U.S. alone were using water supplies in which the level of fluoride is controlled.

The detailed mechanism by which this beneficial action of fluoride on the teeth of young children is effected has been investigated in recent years in several laboratories. It is now usually accepted that the principal agents that lay the teeth open to caries are the organic acids produced locally from adherent carbohydrates by the bacteria growing on the tooth surface. The hard protective enamel is thus dissolved away and the more vulnerable tissue below is open to further and more rapid decalcification and proteolysis. Enamel obtained from areas where the drinking water contains adequate fluoride is more resistant to solution by acids than that from low fluoride areas. The surface enamel from the former areas contains much more fluoride than that from the latter; the hydroxy groups in the hard apatite crystals in the surface enamel are stated to be replaced in the former areas by the harder and more acid-resistant fluorapatite (Leach, 1959). Where the intake of fluoride is adequate this compound is formed in the enamel both before and after the teeth erupt from the gums—an obvious advantage to children who have, from a *very* early age, a sufficient fluoride supply in their ingesta. For this reason a suggested method for fluoride administration, alternative to the addition of sodium fluoride to the general water supply, i.e. that the fluoride should be added to table salt (as in the method in use for many years for protecting against iodine deficiency) is likely to be less effective. Infants may receive little salt in their diet and, in any case, intake of sodium chloride is very variable from one individual to another.

The influence of very low-fluorine diets on experimental rats is to lower their growth rate, to produce more caries and to lead to poorer reproduction than in controls on a normal diet (McClendon & Gershon-Cohen, 1953).

Dental caries is probably not the only human disease associated with fluorine deficiency. Some clinical evidence that low fluorine may be a contributory cause to osteoporosis in the aged has been provided by X-ray findings in reports from U.S. (Leone *et al.*, 1960). In one area with 8·0 ppm in the drinking water the incidence of osteoporosis was 5·6%; with 0·4 ppm the incidence was 10%; in an area with only 0·04 ppm evidence of osteoporosis was found in 42% of the cases; 34% were severe. These observations fit in with the observation that prolonged ingestion of fluorinated water increases the opacity of bones to X-rays, (and also the bones' fluorine content). Treatment of cases of osteoporosis and even Paget's disease (osteitis deformens), where there is acute resorption of bone and a negative calcium balance, with as much as 60 mg of sodium fluoride daily (some 20–30 times the normal ingestion of fluoride by the adult) has yielded positive results in an improvement of the calcium balance and relief from pain in a majority of the patients (Purves, 1962; Sognnaes, 1965).

Some figures may be quoted from studies that have been made of safety factors in water fluoridization (Hodge & Smith, 1963). If the fluoride content of the water is raised to 1·0 ppm, and the diet of the adult is a normal mixed one, the average adult

would have an intake of 2–3 mg fluoride per day (some estimates put the daily intake under these circumstances at only 1·4–1·8 mg), and the average child at about half this amount. The safety factors would then be: for an acute lethal dose 2500–3000; for kidney injury, about 100; for thyroid injury, about 50; for growth retardation, at least 40; for 'crippling fluorosis', 20–80; for mottled enamel a factor of 2–8 or more. As regards reproduction the lowest level at which interference has been reported is 70 ppm in the diet, and that when the fluoride was given for a protracted period to cows.

The figures in the foregoing paragraph assume that the diet consumed is not too bizarre; one composed mainly of dried mackerel, tinned sardines, cotton seed meal and tea would provide the consumer with a daily fluoride intake considerably above the 'normal mixed one' just mentioned. The very high fluorine content of tea—particularly of China tea, ranging from 3 to 400 ppm may possibly be due to the use of a fluorine-containing pesticide. A useful table giving the amounts of fluoride present in various common foods is given in Nikiforuk & Grainger (1964). The milk of cows on a normal diet contains, on the average, only 0·17 ppm, but this can be raised to some 0·30 ppm if the cows' drinking water contains 8 ppm of fluoride.

Ingested fluoride appears to be taken up fairly rapidly from the intestinal contents (Mackle, Scott & Largent, 1942). Even calcium fluoride if given in small amounts is fairly well absorbed. Though an 'insoluble' salt, its aqueous solution at 26°C contains 8 mg F/l, and the administration of a daily dose of 6 mg F as CaF_2 gives almost complete absorption of fluorine.

To the question 'is fluorine an essential micro-nutrient?' we must refer to a definition given in an early paragraph. If 'an essential micro-nutrient' is one 'necessary in very small amounts for the healthy functioning of a living organism . . . including, in the animal . . . tooth maintenance . . . ' then fluorine clearly belongs to this category. Whether an animal could *survive* on a diet free from fluorine is still uncertain. A diet without either of the typical essential trace elements, copper or zinc, would eventually be lethal to any animal; this has not yet been demonstrated for fluorine. The very wide distribution of this element in all common food stuffs and the very great difficulty of eliminating it from every constituent of an experimental diet without making the diet abnormal in other respects have so far prevented a clear-cut answer to the survival question. If fluorine is an essential element in this latter sense, it is quite certain that it will be required in very minute amounts only. What may safely be said is that a sufficiency of fluorine is essential for healthy teeth, and probably for healthy bones also.

Zinc

Zinc, like copper, is well known as an essential micro-nutrient for both animals and plants, and again, like copper, occurs fairly frequently in and about domestic premises in all but the most undeveloped countries, either as bare metal or in alloys or in various other compounds. It is also not infrequent in places where food is manufactured or handled. There is, therefore, some comfort to be gained both from the standpoint of the

food technologist and the food consumer from the fact that, as with copper, there is a wide gap between the minimal level of zinc needed for normal health and nutrition, both in man and in farm animals, and the toxic level. For zinc the gap is wider than for Co, F, Mo or Se. Further, the average mixed diet of the human adult provides ample zinc—of the order of 8–15 mg daily—and either human or cows' milk contains about enough zinc (3–5 mg/l) to supply the needs of the infant or to supplement the needs of the normal child. It has been stated (Underwood, 1962, p. 179) that 'an uncomplicated dietary deficiency of zinc has never been observed in man'. Further, in view of the high tolerance of most experimental animals to large and continued dietary administration of zinc—of the order, for example, of 1000 ppm of the diet—it seems most unlikely that, outside special industrial circumstances, zinc poisoning in man or in farm animals will occur. In experimental animals, toxic symptoms begin to be shown when the diet contains the very substantial proportion of some 3000 ppm or above. These symptoms may not, in some cases, be due to zinc poisoning as such but may arise from the effect of high intake of zinc in depressing copper utilization.

In normal animal or plant tissues zinc is present combined, or closely associated, with protein, and that in distinctly larger quantities than other 'trace' elements such as Co or Cu. In a very few of these tissues it is present in quantities that approach or even exceed the amounts of the *major* tissue constituents. Thus zinc is highly concentrated in the choroid of the eye; this tissue in the fox has been reported to contain as much as 6·9% (69,000 ppm) on the dry weight of the tissue (Weitzel & Fretzdorf, 1953).

The skin and hair of man and other mammals contain zinc in much smaller quantities—of the order of 200 ppm—but still, for a 'trace' element, a high concentration. The islets of Langerhans, in the pancreas, are also rich in zinc, which is excreted from this gland in the pancreatic juice. Here it acts as co-enzyme to peptidase (Vallee *et al.*, 1960). It has been stated—and contradicted—that in the diabetic pancreas, where insulin secretion is defective, the zinc content is reduced. The element also occurs, with no obvious function, in fairly large amounts in the prostate gland and its secretions, in whole semen and in spermatozoa.

Knowledge of the specific part played by a trace-element in metabolism begins to be more clearly defined when that element can be shown to occur as a normal constituent of a physiologically-active tissue component such as a vitamin, a hormone or an enzyme. Zinc was shown, as long ago as 1938, to form an integral part (0·33%) of the molecule of the enzyme carbonic anhydrase which is present in mammalian red cells in large amounts; most of the zinc in these cells is present as part of the enzyme. The enzyme is also to be found in the reproductive tract of the laying hen. In both of these tissues its known function is to increase the speed of both phases of the reversible reaction between carbonic acid, H_2CO_3, on the one hand, and CO_2 and water on the other. In the blood the enzyme assists in the quick liberation of CO_2 through the lungs into the expired air; in the fowl it is concerned with the laying down of the calcium carbonate

that forms the main part of the egg shell. It is also found in high concentration in the brain, where its function is, at present unknown (Ashby, Garzoli & Schuster, 1952). In the last few years other enzymes have been found to contain zinc in their molecule; amongst these are certain peptidases and also alcohol dehydrogenase, with activities remote from those of carbonic anhydrase. Alcoholic cirrhosis of the liver and disturbances in zinc metabolism are closely associated.

Carbonic anhydrase has important functions in the green plant where, in the chloroplast, it is closely associated with the second phase of the reversible reaction just mentioned, namely the uptake of CO_2 from the air—an early stage in the photosynthetic process upon which all mammalian life ultimately depends. A few species of plants, apart from the small amount of metabolically active zinc in their carbonic anhydrase, accumulate in their leaves enormous stores of zinc to which it is difficult to assign any useful function. Such accumulations, which, like the immense amounts of selenium present in *Astragalus* species, appear to be so much lumber, may exceed, in percentage content of the tissue, even those in the mammalian choroid. *Thlapsi calaminare* may have as much as 16% Zn in its ash.

Plants find increasing difficulty in taking up zinc (and copper) from soils containing a high quantity of phosphate or calcium, though the uptake of molybdenum may thereby be stimulated—another example of the important interrelationship between the micro and macro plant nutrients.

Experimental zinc deficiency symptoms can fairly readily be induced in the rat, the fowl and the pig by appropriate modification of the diet (galvanized iron cages or buckets, etc., have to be avoided!). The minimum requirement of zinc for maintenance of health, taking into account the small amount still remaining in the experimental diet, can then be determined. This minimum for young pigs would appear to be in the neighbourhood of 40–50 ppm of Zn on the dry weight of the diet, rather less than this for poultry and probably less again for calves (Mills *et al.*, 1966). The symptoms which appear in zinc-deficient animals include excessive salivation, retardation of growth, thickening of the skin ('parakeratosis') loss of hair and bone defects, symptoms increasing in severity as the proportion of zinc in the diet is reduced. In the fowl there is poor hatchability of the eggs. But even in severe zinc deficiency the amount of carbonic anhydrase in the blood is not depressed.

As mentioned above, these findings are derived from animal experiments where diets are under more careful control than is possible in the field. Where, either in the field or on the farms, animals receive a diet adequate in protein, zinc deficiency is unlikely and, as regards cattle, it has not been reported, as yet, under British conditions. Probably the minimum zinc requirement of cattle is not far removed from that of poultry, say 30–40 ppm in the diet. This figure is apparently not reached in parts of Holland, Norway and Finland, where mild zinc deficiency, resulting in a fall in milk production, has been observed. This may well be a high calcium effect. An addition of zinc carbonate (100 ppm of the diet) is stated to improve the growth rate of weanling

pigs. It would be interesting to know whether this zinc effect, and the beneficial effect of 250 ppm of copper (mentioned previously) were synergistic.

In experimental work in the animal nutritional laboratory with zinc—or indeed with any other trace element—the availability of the micro-nutrient is, as with plants, often found to vary greatly with changes in the amounts of the major nutrients such as phosphorus, calcium, potassium and (with animals) protein in the diet, and also with the presence of metal binding, chelating and precipitating agents. Phytic acid, which retains zinc very firmly, diminishes its uptake from the gut. Soya bean meal, unless autoclaved, is known to reduce the availability of dietary zinc to the fowl, but this can be restored by the addition to the soya-containing diet of the powerful chelating agent ethylene diamine tetracetic acid which, unlike phytic acid, keeps the zinc in solution. Other chelating agents, known to be present in liver extract and in 'distillers direct solubles' may, similarly, assist in the uptake of trace elements as well as of other mineral constituents of the diet.

Applications in crop husbandry

The close relationship, recapitulated in the foregoing pages, between trace elements and biological systems provides another illustration—if such were needed—of the way in which living organisms have taken physiological advantage of apparently insignificant features of their environment (see Table 3), but by doing so have rendered themselves

TABLE 3. Daily requirement of trace metals and proportion in soils

Micro-nutrient	Daily requirement (human adult) (mg)	Proportion of earths crust (%) (Nyholm, 1967)	Mean value (ppm of dry soils) (Gerber, 1965)
Fe	12–15	5·1	38000
Mn	3–5	0·09	850
Zn	10–20	0·020	50
Cu	1–3	0·010	20
Co	$<0{\cdot}1$	0·0012	8
Mo	*Very* small	0·00015	2

The (presumptive) daily human requirement of any one of the metallic micro-nutrients is roughly in proportion to the amount of that metal in the earth's crust or in the soils. Zn is probably an exception, though the figure for daily requirement shown above may be too high.

more vulnerable to changes in it. At a very early stage in the development of life on earth the primeval forerunner of the chloroplast must have picked up small quantities of copper and zinc to form essential parts of its enzymic equipment, and must also have accepted traces of Mn, Mo and Cl as guarantors of its normal function. Today, should any one of these become seriously deficient in the plants themselves or in a locality in

which the plants are endeavouring to grow, the whole ecosystem becomes deranged.

The increasing realization in the agriculturally advanced countries of the part played in crop production by micro-nutrients has begun to make itself felt in practice, though there is still, in many of these countries, little information as to the trace element status of substantial areas of their cultivated soils. Without this information, though other agronomic factors obviously have an important influence, an optimum nutrient balance, with its beneficial effects on food quality as well as quantity, between the plant nutrients as supplied in the major soil fertilizers and the essential trace minerals, can hardly be achieved. In the less developed countries, the cautious introduction of macro-nutrients to soils which have been grossly impoverished by generations of unfertilized cropping will obviously be of very great advantage to crop or grassland production, but it would seem highly desirable that it should be preceded by an examination of the amount both of available trace elements as well as of the major nutrients in representative soils. In many districts a significant improvement in crop yields per hectare, in animal production and in human nutrition could be quickly and inexpensively achieved by *dressings of the deficient micro-nutrients alone*, though eventually a balance between the various nutrients, major and 'trace' would be the natural objective.

The important questions of: (a) the availability to the plant of micro-nutrients in the soil, and (b) the availability to man or other animals of such trace elements in foods of plant or animal origin, have been only briefly touched on in the present review. A useful discussion on aspects of (b) may be found in a Symposium on the Availability of Minerals in Foods of Plant Origin (1965).

To sum up, it is clear that the first impact of deficiency or excess of the essential micro-nutrients is likely to be on the plant, vulnerable because it must live or die in the exact locality in which its seed was accidentally or purposely introduced. The farm animal living in a restricted area or on a restricted diet is, particularly in an agriculturally primitive country, somewhat less vulnerable. The small cultivator or impoverished town dweller in such a country, even if he is often on a monotonous diet short of the protein with which the trace metals are usually associated, is rather unlikely to run into danger from lack of these, though his young children may. Whether, as has been occasionally suggested (Schülte, 1964; Gerber, 1965), certain chronic human ailments can be mitigated by treatment, with amounts larger than occur in the normal, mixed dietary, of this or that trace element (other than iron, which for present purposes has been excluded from this category) has not yet been established.

Finally, having regard to their mixed diets of varied origin, it can be assumed that the inhabitants of developed countries, children or adults, are virtually safe from trace metal deficiencies or excesses. They are, however, not so safe, under present circumstances, from deficiencies in fluorine or iodine.

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The addition of iron to flour

I. The solubility and some related properties of iron powders including reduced iron

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Introduction

Since 1953 there has been a statutory minimum iron content for flour of 1·65 mg/100 g. White flour of about 70–72% extraction, which has a natural iron content of 1–1·2 mg/100 g must, therefore, be enriched in order to reach the statutory minimum. The most recent Order—*The Bread and Flour Regulations* (Statutory Instruments, 1963)—lays down that the iron addition shall be in the form of ferric ammonium citrate (current B.P. or B.P. Codex) or reduced iron made by the action of hydrogen on ferric oxide. Another form of iron powder is that produced by the electrolysis of an iron salt. Both forms have been included in the present experiments since when they dissolve in dilute hydrochloric acid or gastric juice they both produce the same primary material in the absorption process, *viz.* ferrous chloride.

At present much of the flour in this country is enriched with reduced iron. Recently, however, Elwood (1963, 1965) from studies on a group of adult patients in a mental hospital, has raised doubts about the value of the present level of enrichment with this form of iron. Following on this publication we have studied some of the physical properties of different commercial samples of reduced iron, particularly their relative solubilities in gastric or simulated gastric juice. Our results are described in this paper.

Reduced iron

The literature on the chemistry and technology of the production of reduced iron is scanty. It can be obtained as a black powder by reducing one of the oxides (magnetite Fe_3O_4 or haematite Fe_2O_3) in a current of dry hydrogen. The temperature of reduction, according to Mellor (1932), may be from about 350° to 1150°C the lower the temperature the longer the time for the reduction. Below about 600°C the resulting finely divided powder is pyrophoric and when exposed to the air will oxidize extremely rapidly, and may take fire. At high temperatures the particles of iron are sintered, i.e. they agglomerate to form larger particles. It would be expected, therefore, that the

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particle size, the porosity of the particles, the surface area per gram and in turn the solubility of a sample of iron powder all depend on the conditions and particularly in the case of reduced iron the temperature of manufacture. *The Bread and Flour Regulations* (1963) do not suggest any limiting temperature but the B.P. Codex 1934 mentions that over-heating produces a coarse preparation and suggests heating to 'dull' redness.

Leadbeater, Northcott & Hargreaves (1947) carried out an extensive examination of twelve reduced iron powders and thirteen powders prepared electrolytically. Certain of their results are shown in Table 1.

TABLE 1. Type of powder

Property	Reduced iron		Electrolytic	
	Range	Average	Range	Average
Iron content (%)	96·7–98·7	98·0	98·5–99·6	99·2
Median size (μ)	6–95	64	47–114	66
Particle porosity (%)	0·19–3·31	1·97	0·78–3·81	2·13
Specific surface (m^2/g)	0·448–5·16	1·036	0·265–1·149	0·534

These authors did not measure solubility, but their results demonstrate the variable nature of commercial samples of iron powder in either category.

Experimental

We have examined two samples of reduced iron manufactured in the U.S.A. (A1 and A2),* five samples of reduced iron made in this country (B1–B5), four samples of electrolytic iron also made in this country (C1–C4) and one of reduced iron (L) specially prepared in the laboratory at a low temperature and supplied to us by Dr Elwood.

Determination and recovery of iron

Throughout, iron was determined as the *o*-phenanthroline complex after solution in 1 : 1 concentrated HCl or evaporation of solutions, ashing and solution of the ash in 1 : 1 HCl (Pringle, 1946).

The recovery of metallic iron. In some of the experiments iron metal was extracted magnetically from suspensions or digestions. A U-shaped magnet 5 × 3·5 × 3·5 cm

* A 1 was sent over by one of us (T.M.) from the U.S.A. in 1944. Some was used for the enrichment of the flour used in the Widdowson–McCance investigation (Widdowson & McCance, 1954) and the remainder kept in a stoppered bottle. Sample A2 from the same firm, but now made for them by an outside company, was sent over in 1964.

was wrapped in polythene film and passed, systematically and repeatedly, through the liquid in a shallow dish. After rinsing, the magnet was transferred to a dish of water or with flour suspensions, petrol ether, removed from the polythene wrapper and the metal brushed from the latter into the liquid. Preliminary experiments showed that the efficiency of recovery of weighed amounts of iron powder added to homogenates and suspensions was 90% approximately. Iron A1 because of its extreme fineness was exceptional in that the recovery was only 50%. These factors were applied to the results.

Recovery of iron from bread and flour. The crumb was broken down either: (i) in water in a laboratory homogenizer for 15 min, or (ii) by digesting a 5-g crumb with 100 mg papain and 100 mg fungal amylase in 100 ml water (pH about 5) for 18 hr at 37°C followed by homogenizing for a short period. Flour was suspended in CCl_4.

Determination of solubility

(a) In vitro. The determination of solubility was very sensitive to conditions of temperature, agitation, type of vessel, etc.

All the samples contained at least 95% iron and were almost completely soluble in 0·1 N HCl given sufficient time, e.g. in some cases 24 hr.

HCl alone. Quickfit stoppered tubes MF/24/3/8 of 85 ml capacity containing 4 mg iron powder and 50 ml 0·1 N HCl were rotated end over end at 17 rev/min in a constant temperature room at 23°C for times varying from 30 min to 2 hr.

HCl in the presence of pepsin. Two milligrams iron powder, 100 mg of pepsin and 25 ml 0·1 N HCl were maintained at 37°C for 2 hr. The conditions were chosen to resemble more closely normal gastric digestion. The reaction was carried out in conical flasks which were gently swirled at 30-min intervals. Pepsin B.P. containing lactose as diluent was used in these experiments. Both this product and pure crystalline pepsin depressed the solubility of iron powder in 0·1 N HCl.

(b) In vivo. Bread was baked from flour containing 21 ppm of the different samples of iron powder and fed to rats as described in the following paper. Food intake was measured daily and the metallic iron excreted was also determined daily by recovery from the faeces (homogenized with water) from all the rats on a particular bread. The experimental period was from 1 to 2 weeks, six rats being included for each experiment. At the end of the experimental period the rats were given non-enriched bread but the faeces were analysed for excreted metallic iron for a further 2 days.

Discussion

The results of the solubility tests are given in Table 2.

The relative solubilities by all three methods are in good agreement and the fact that the figures for simulated gastric juice are in accord with those from the digestion

TABLE 2. Solubility of iron powders under different conditions

Sample	0·1 N HCl 30 min at 23°C (%)	0·1 N HCl, pepsin 0·4% 2 hr at 37°C (%)	*In vivo* in the rat (%)
A1	93	82	90
			81
A2	37	28	39
B1	30	20	19
			15
B2	—	23	—
B3	39	27	36
B4	34	27	27
B5	30	—	—
C1	53	69	58
			42
C2	44	53	—
C3	42	38	—
C4	52	58	60
L	100	99	100

of the iron enriched breads by the rat suggests that the bread as such does not interfere with the solution and absorption of its added iron.

The A, B and L samples of reduced iron vary widely in solubility. There is also a marked difference between the two samples A1 and A2 but we have been unable to obtain details of the manufacture of either sample. The C samples of electrolytic iron powder, generally have a higher solubility than the B samples. Sample L, completely soluble in gastric juice, was freshly prepared at a low temperature in the region of 500°C. As would be expected, however, it oxidized rapidly and after a few weeks (in an envelope) at ordinary temperature its solubility in pepsin–HCl had fallen from 99 to 53%. Incidentally the solubility of a sample of Fe_2O_3 was found to be only 5%.

Solubility would be expected to go hand in hand with susceptibility to oxidation. Thus samples of A1 and B4 were stored at room temperature in a water saturated atmosphere and the figures in Table 3 for solubility obtained.

These of course were exaggerated conditions, never likely to be encountered in practice.

On the other hand a low solubility of a particular sample of iron powder is not necessarily due to surface oxidation. Thus sample B1 was heated in a current of hydrogen for 30 min at 600°C. It was then cooled in hydrogen and its solubility determined immediately in pepsin – HCl at 37°C. It was found to be 18% compared with the original figure of 20%.

TABLE 3. Solubility in 0·1 N HCl, pepsin 0·4%, 2 hr at 37°C

	A1(%)	B4(%)
Initially	82	27
After 45 hr storage	61	23
After 140 hr storage	53	25

Particle shape and size

A marked difference in particle shape was observed between the iron powders prepared electrolytically and by reduction. The former were mainly prismatic in shape with sharp angular outlines typical of a shattered crystalline mass. The latter were in general spherical with smooth rounded outlines, samples B1–B5 and A2 being typical sintered aggregates, much larger in size than samples A1 and L of greater solubility.

As would be expected, other things being equal, a rough relationship does exist between size and solubility. This was shown on a coarse and fine fraction from sample C1:

	Solubility (%)	
	Coarse	Fine
0·1 N HCl, 0·4% pepsin, 2 hr at 37°C	57	72

Nevertheless, an analysis of particle size distribution, using the Sartorius sedimentation balance, did not show any clear cut relationship between size distribution and solubility for powders prepared in different ways. Surface area per gram of powder was, however, more revealing. It ranged from 102 m^2/g for the very soluble powder L to 0·1 m^2/g for sample B1. The figure for A1 was 4·8. This work on particle size and surface area was carried out by Dr J. Butcher and will be published separately.

Changes in solubility of added iron during the storage of flour

Direct experiment showed that more than 90% of the iron powder added to flour could be recovered unchanged from the resulting bread. Table 2 also confirms this finding.

In the United Kingdom white flour is enriched by the addition of a 'master mix' to the flour stream at the rate of 1 oz per sack of 280 lb of flour. The master mix has the following composition:

	% by weight
Vitamin B_1	0·74
Nicotinic acid (or amide)	3·53
Reduced iron	3·14
Flour diluent	92·59

It was found that the solubility of the iron (B4) extracted from a sample of master mix freshly made and after 15 months storage was the same.

On the other hand in the case of flour enriched with the highly soluble iron powder (A1) the solubility in pepsin—HCl of the powder, extracted after storage for 14 weeks had fallen by about 10%.

All these results fit into a logical picture. From the point of view of the enrichment of flour with reduced iron it is clear that the desirable standard for this form of iron should be one combining the maximum solubility or ease of solution consistent with the minimum tendency to become oxidized during its storage either in air or in flour. The temperature to which the iron oxide is submitted either before or during the reduction process is probably the critical factor.

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The addition of iron to flour

II. The absorption of reduced iron and some other forms of iron by the growing rat

J. J. C. HINTON* AND T. MORAN†

Introduction

The previous paper showed that reduced iron is not a standard product in respect of the ease with which it dissolves in dilute hydrochloric acid, in simulated gastric juice or in the stomach of the rat. This work has been extended to measurements of the actual total absorption of iron by the rat, from different samples of reduced and electrolytic iron powders, and also from other forms of iron that are used or have been suggested for the enrichment of flour.

Experimental

The different forms of iron were incorporated into a bakers grade flour containing no chalk, vitamin or other additions, and this flour was baked into bread. A small and variable amount of metallic iron (ranging in seven samples from 0·05 to 0·43 mg/100 g) was present as a contaminant from the steel rolls in the mill.

Normal iron enrichment of flour in this country is 7 ppm, but generally we have used 21 ppm in an attempt to reduce the experimental errors which beset this type of work. Furthermore, it is a reasonable level since to reach the iron content of whole wheat would require about 30 ppm. Also in the U.S.A. the level in practice ranges from 19 ppm (minimum) to 27 ppm (maximum).

Care of the animals

Female rats, which in each experiment were matched from the same litters, were housed, three to a cage, in specially made aluminium cages. Six rats (twelve in Experiment 3) were used for each diet and in every case a 'control' diet was included in which the rats were fed non-enriched bread.

The rats (Norwegian strain) were transferred at weaning (3–4 weeks old) to a bread diet made with flour containing no added iron (Experiments 1–9) until the start of the experiment.

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In the final experiment (Experiment 10) the rats were made iron deficient by transferring them at weaning to a milk diet for 3 weeks. They were then fed the experimental bread diets. The liquid milk diet of Elvehjem & Kemmerer (1931) was inadequate to maintain health, excessive diarrhoea and dropsical swellings of the feet occurring after 4 or 5 days. The milk diet of McCall *et al.* (1962), however, slightly simplified as shown below, and fed *ad libitum*, supported growth and health during the 3-week experimental period.

Diets

The bread was air dried to about 10% moisture and coarsely ground.

	Bread diet	Milk diet
Dried crumb	87·5%	—
Arachis oil	5·0%	—
Salt mixture (Jones & Foster) (omitting iron)	2·0%	—
Liver powder	0·4%	—
Choline chloride	0·2%	—
L-Lysine hydrochloride	0·3%	—
Vitamin B_1	0·5 mg/100 g	1·5 mg
Vitamin B_6	0·5 mg/100 g	1·5 mg
Nicotinic acid	0·5 mg/100 g	1·5 mg
Riboflavin	1·0 mg/100 g	3·0 mg
Calcium pantothenate	2·5 mg/100 g	7·5 mg
p-Aminobenzoic acid	15·0 mg/100 g	45·0 mg
Folic acid	—	1·5 mg
Sucrose as diluent for the vitamins to 100%		
Dried skim milk	—	50·0 mg
Liquid milk	—	1000 ml

The normal supplements of vitamins A, D and E were given orally at weekly intervals.

Intake of food, fed *ad libitum*, was recorded daily and the faeces were collected at the same time. Distilled water was provided; urine was not collected, the amount of iron excreted in this form being negligible compared with that in the faeces.

The experimental diet was fed for approximately 1 week followed by a period on non-enriched diet. The iron excreted, recorded in Tables 1 and 3, included a period of 2–4 days after the termination of the experimental diet by which time it had fallen to a relatively constant value.

Determination of iron

Iron was determined as the *o*-phenanthroline complex as described in the previous paper.

Diets and other dry samples were ashed directly.

Metallic iron was first extracted from the faeces* (the total for any one group) by the magnetic method described in the previous paper, the faeces being soaked in water for 2 or 3 hr and suspended in water using a laboratory homogenizer. In the next step the suspension was again agitated and any ingested aluminium allowed to settle for a few moments; an aliquot of the suspension was then withdrawn for the determination of non-metallic iron. The aluminium interfered because of its small iron content (approximately 0·5%) and its tendency to precipitate phosphate during colour formation.

Determination of haemoglobin

In 0·02 ml blood taken from the tail as cyanmethaemoglobin using the International Standard procedure (C. Davis Keeler Ltd, 47 Wigmore Street, London, W.1).

Results

The forms of iron enrichment included six of the samples of iron powder dealt with in the previous paper, ferrous sulphate, sodium iron pyrophosphate and ferric ammonium citrate (green). As a matter of interest wholemeal flour containing no added iron was included, but in one experiment only.

The green citrate was used in preference to the brown because experiments have shown that it is not so conducive to the production of rancidity in flour stored for long periods (Martin & Halton, 1964).

Normal rats

In nine experiments, the results of which are given in Tables 1 and 2, the animals commenced with iron status only a little below normal (blood haemoglobin averaged 12 g/100 ml) so that their requirements would, therefore, depend mainly upon growth. Table 1 gives the intake and excretion of total dietary iron and in Table 2 the iron retained is expressed as per cent of the total iron ingested. Fig. 1 shows the course of a typical experiment.

Anaemic rats

In a final experiment (Experiment 10) two samples of reduced iron, one of high (L) and the other of low solubility (B2), ferrous sulphate and ferric ammonium citrate were compared in partially anaemic animals. At the start of the experiment the mean haemoglobin content was 8·7 g/100 ml blood with no significant differences between the means of the rats on the different diets. Table 3 gives the figures for iron intake and retention.

* To provide some of the figures given in Table 2 in the preceding paper.

TABLE 1. Retention of iron by normal rats from enriched bread

Experiment	Enriched with	Period on experimental diet (days)	Average age of rats (weeks)	Per rat per day			
				Total food intake (g)	Total iron intake (mg)	Total iron excreted (mg)	Total iron retained (mg)
1 (a)	NE	9	9	11·7	0·152	0·112	0·040
(b)	7 ppm sample B4	9	9	11·4	0·205	0·136	0·069
(c)	21 ppm sample B4	9	9	13·5	0·304	0·236	0·068
2 (a)	NE	11	15	12·7	0·157	0·117	0·040
(b)	70 ppm sample B4	11	15	12·9	0·609	0·564	0·045
3 (a)	NE	8	10	14·4	0·214	0·188	0·026
(b)	30 ppm sample A 1	8	10	13·1	0·471	0·400	0·071
(c)	Wholemeal	8	10	12·6	0·432	0·355	0·077
4 (a)	NE	9	8	11·7	0·155	0·092	0·063
(b)	21 ppm sample A 1	9	8	12·6	0·353	0·242	0·111
(c)	Ferric ammonium citrate	9	8	13·4	0·398	0·182	0·216
5 (a)	NE	10	5	11·1	0·153	0·098	0·055
(b)	21 ppm sample B1	10	5	11·5	0·291	0·232	0·059
(c)	21 ppm sample C1	10	5	11·7	0·297	0·232	0·065
6 (a)	21 ppm sample B1	5	5	9·5	0·210	0·171	0·039
(b)	21 ppm sample C1	5	5	10·1	0·213	0·154	0·059
7 (a)	NE	8	8	12·8	0·168	0·112	0·056
(b)	Sodium iron pyrophosphate	8	8	13·0	0·372	0·262	0·110
8 (a)	NE	7	4	8·2	0·116	0·088	0·028
(b)	Ferric ammonium citrate	7	4	8·0	0·245	0·160	0·085
(c)	Ferrous sulphate	7	4	8·2	0·287	0·214	0·073
9 (a)	NE	9	5	9·5	0·134	0·091	0·043
(b)	21 ppm sample C4	9	5	9·7	0·282	0·209	0·073
(c)	Ferric ammonium citrate	9	5	10·8	0·292	0·177	0·115
(d)	$FeSO_4$	9	5	10·7	0·271	0·184	0·087

NE signifies 'no enrichment'. The sample numbers refer to iron powder samples numbered as in the previous paper. The three salts were added in a concentration to correspond to 21 ppm of iron.

TABLE 2. Iron retained as per cent total iron ingested

Experiment	NE	Sample B1	Sample B4	Sample C1	Sample C4	Sample A1	Ferric ammonium citrate	$FeSO_4$	NaFe pyrophosphate	Wholemeal
1	26		34(7) 12(21)							
2	25		7(70)							
3	12					15(30)				18
4	40					32(21)	54			
5	36	20		22						
6	—	19		28						
7	33								29	
8	24						35	25		
9	32				26		39	32		
Average	28									

Figures in parentheses indicate degree of enrichment (ppm); in all the other cases, except for those marked NE, it is 21 ppm.

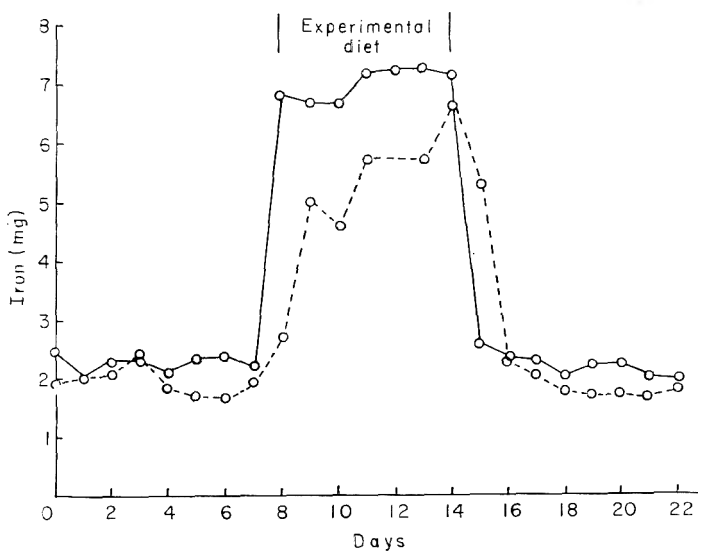

FIG. 1. Ingestion (———) and excretion (– – – –) of total iron in a typical experiment (Experiment 3b).

TABLE 3. Retention of iron from enriched bread by anaemic rats

Enrichment (21 ppm)	Experiment No.	Period on experimental diet (days)	Average age of rats (weeks)	Per rat per day					Iron retained as % of	
				Total food intake (g)	Total Fe intake (mg)	Total Fe excreted (mg)	Total Fe retained (mg)	Added Fe* retained (mg)	Total intake	Added iron
NE	10A	15	7	13·0	0·182	0·112	0·070	—	38	—
Sample B2	10B	18	7	11·8	0·403	0·298	0·105	0·042	26	18
Sample L	10C	13	7	11·9	0·375	0·244	0·131	0·067	35	32
Sulphate	10D	18	7	12·1	0·385	0·235	0·150	0·085	39	39
Citrate	10E	17	7	14·2	0·438	0·238	0·200	0·124	46	52

* Obtained from the difference between non-enriched and enriched diets after making allowance for the difference in food intake.

The haemoglobin content of the blood of each rat was also determined at intervals during the course of the experiment. Unfortunately our stock of iron powder L was small and was exhausted within 12 days. Table 4 gives the changes in haemoglobin content during the first 11 days whilst the individual changes in the four groups are shown in Fig. 2.

TABLE 4. Increase in blood haemoglobin values of anaemic rats

Diet	Haemoglobin levels at 11 days (g/100 ml)
Non-enriched	9·6
Reduced iron B2	9·4
Reduced iron L	12·5
Ferrous sulphate	13·35
Ferric ammonium citrate (green)	13·65

Discussion

In this country, on the average, the iron added to bread supplies about 10% of our total intake. In the present experiments at the levels of enrichment corresponding to 70, 30, 21 and 7 ppm the percentages of the total intake due to added iron were approximately 80, 70, 60 and 30%, respectively. The results might, therefore, be expected to emphasize any marked differences in the availability of the different forms of added iron. Although it was not feasible to do the iron balance studies on each rat, the

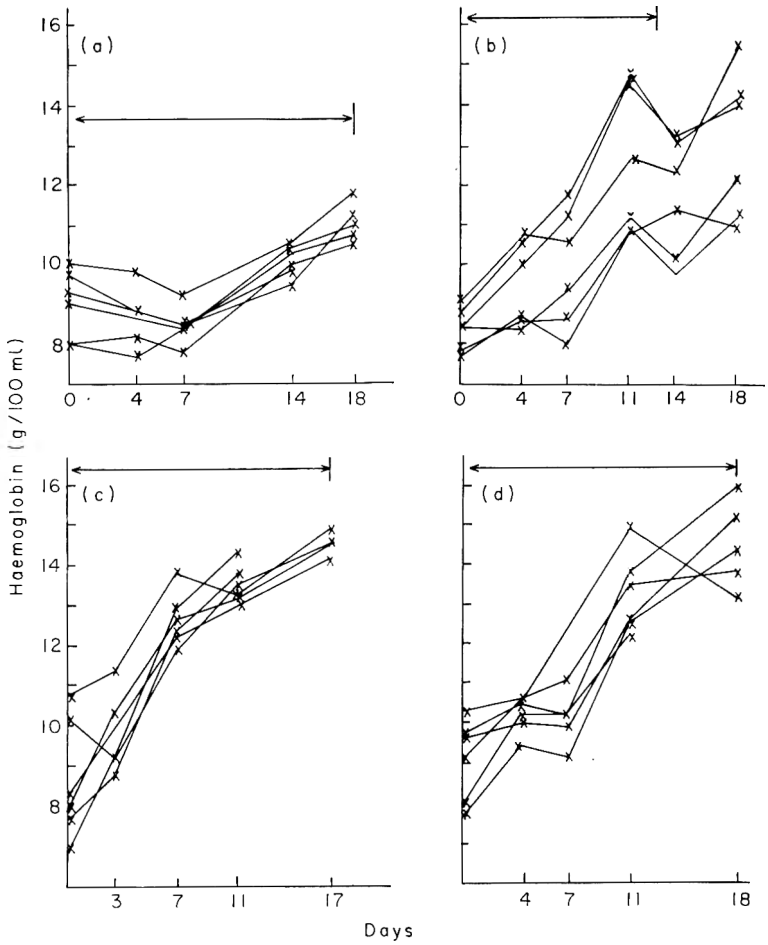

FIG. 2. Changes in haemoglobin content in four groups. (a) Reduced iron 37% solubility, Experiment 10(b); (b) reduced iron 100% solubility, Experiment 10(c); (c) ferric ammonium citrate, Experiment 10(e); (d) ferrous sulphate, Experiment 10(d). Arrow indicates period of enriched diet.

averages for each group, in comparable experiments, indicate that in general the different forms of iron when baked into bread were all well absorbed. The experiments, therefore, provide further evidence in favour of the enrichment of bread-making flour with iron. Also another interesting result is the comparative constancy of the absorption of iron from non-enriched bread averaging 28%. Moore *et al.* (1944) claim that there is a species difference in iron absorption. Even so it would be exceptional if the present experiments did not give pointers applicable to man.

Statistical examination of the figures for the haemoglobin content in the individual animals at 11 days showed no significant difference between ferric ammonium citrate

and ferrous sulphate. On the other hand, that between citrate and reduced iron L just reached the 5% level of significance, whilst that between ferrous sulphate and L was less significant. The broad conclusion is that any differences between these three sources of iron are unimportant. By contrast the haemoglobin contents for citrate, sulphate and iron powder L are all significantly higher ($P < 5\%$) than those for iron powder B2.

There are the usual apparent paradoxes found in most published papers on this problem. Thus: (i) In man it is generally accepted that ferrous salts are much more readily absorbed than ferric salts both by normal and by anaemic patients, cf Keele & Neil (1961). Yet for the rat, ferric ammonium citrate is as well absorbed as ferrous sulphate. Likewise, Steinkamp, Dubach & Moore (1955) found the same degree of absorption with single doses of radioactive reduced iron, ferrous sulphate, ferric orthophosphate and sodium iron pyrophosphate baked into bread and fed to thirty-two healthy young men and women. These results also seem to be incompatible with the practice of giving a supplement of ascorbic acid in iron therapy because of its reducing action. (ii) Despite its high content of phytates the absorption from wholemeal bread is sensibly the same as that from white bread enriched to wholemeal level with the relatively soluble reduced iron A1. This however is in accord with the recent work of Cowan *et al.* (1966) showing that dietary phytate has no effect on iron absorption in the rat. (iii) The poor performance of iron powder B2. Although the apparent drift downwards in the first 7 days is within the experimental error the overall increase in haemoglobin content over the full experimental period of 18 days is considerably less than would be expected.

Acknowledgment

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The baking properties of pasteurized whole egg

R. A. KNIGHT, K. MEARS, T. L. PARKINSON AND J. ROBB

Summary. A comparison was made of raw and pasteurized egg from twenty-eight processing plants. The average composition of the raw egg agreed closely with published data on commercial shell egg but the pasteurized samples contained slightly less fat and total solids.

Pasteurization resulted in a small, but statistically significant, loss of baking quality in 'sponge batter' sponges but in 'all-in' sponges the mean difference in performance of raw and pasteurized egg was not significant. The mean performance of pasteurized egg in baked custards was slightly inferior to that of raw egg, and a small proportion of the samples examined performed rather poorly in this respect. No significant differences were observed between the baking characteristics of pasteurized first and second quality egg.

When raw and pasteurized egg were stored for about 2 years at a temperature of 12–16°F, the raw egg maintained its quality but the pasteurized egg deteriorated after about 30 weeks storage when used to make products sensitive to egg quality.

However, even after 2 years storage the pasteurized samples gave satisfactory results in other baked products.

No significant changes in performance were observed over a period of 4 days when pasteurized egg was held at 38–40°F without continuous agitation.

Separation of fat occurred when liquid pasteurized egg was kept or transported without agitation and this led to a small loss of baking quality.

Raising the pasteurization temperature above 148°F resulted in a further small loss of baking quality. The additional loss resulting from raising the temperature to 152°F was comparable in extent to that caused by normal pasteurization.

The homogenization of thawed pasteurized egg of poor baking quality improved its performance in sponges and baked custards.

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Introduction

The *Liquid Egg* (*Pasteurization*) *Regulations* (1963) stipulate that liquid egg used as an ingredient in the preparation of food must be pasteurized by holding at a temperature not lower than 148°F (64·4°C) for at least $2\frac{1}{2}$ min. Pasteurized liquid egg is supplied to the bakery trade in the chilled or frozen state for use in flour confectionery, and it is necessary to know to what extent the pasteurization process affects the quality of cakes and other products made from liquid whole egg. Certain baking tests are more suitable for this purpose than others and these have been applied to a direct comparison of a number of raw and pasteurized samples, each pair taken from the same batch, and to a study of the influence of storage and other factors on the baking properties of pasteurized egg.

Review of the literature

Factors affecting baking quality

Using methods developed by Platt & Kratz (1933) for measuring and recording the characteristics of sponge cakes, King and co-workers (King, Morris & Whiteman, 1936a; King, Whiteman & Rose, 1936b) concluded that there was no apparent relationship between certain physical and chemical properties of the egg (pH, carbon dioxide content, total solids and viscosity) and the quality values of the cakes.

Glabau (1950a, b) examined the use of frozen eggs in the manufacture of sponge cakes and layer cakes. For making sponge cakes he took samples of egg from the outer edge, the intermediate portion and the core of the can, and compared them with a sample of well-thawed egg representing the whole can. He obtained some differences in the characteristics of the finished cake and concluded that a high solids content was desirable for the production of a good sponge cake. Vander Voort (1952) made sponge cakes with eggs having solids contents of 26·1, 24·2 and 21·9%. As the solids contents decreased the batters required longer beating times and the cakes had smaller volumes.

Cornford *et al.* (1960) compared the quality and baking performance of shell eggs from free-range and battery hens. The only really marked difference was in colour, which was much paler in the battery eggs; otherwise the differences were small and of no commercial significance, e.g. the average values for sponge cake volumes differed by about 3%. Five tests were carried out at monthly intervals, and it was observed that quality and performance tended to vary slightly from month to month to an extent that was greater than any difference between the two types of eggs. The total solids contents of the eggs varied between 25·8 and 26·6%, with a mean value of 26·3%.

Coppock (1962) described experiments in which whites and yolks were separated and then recombined in various proportions, sponge cakes being baked from the different mixtures. It was found that cake volumes decreased considerably when the ratio of white to yolk was greater than 2 : 1. The best cake was obtained with a ratio of 1·8 : 1 (i.e. 64·5% of white), but mixtures containing higher proportions of yolk

still gave high cake volumes. The natural ratio of white to yolk in eggs was stated to vary between 1·6 : 1 and 2·3 : 1.

Using the average values for total solids of yolk (50·47%) and white (12·21%) given by Mitchell (1932) for 'commercial fresh eggs' the white/yolk ratios in Coppock's paper can be transformed to average total solids contents, viz.

White/yolk ratio	Average total solids content (%)
1·6: 1	26·9
1·8: 1	25·9
2·0: 1	25·0
2·3: 1	23·8

These figures are worth noting in view of the results of Vander Voort (1952) and of the satisfactory cake volumes reported by Cornford *et al.* (1960) when using shell eggs of 25·8–26·6% solids content.

An interesting study of the functional properties of egg white proteins was carried out by MacDonnell *et al.* (1955). They separated 2 litres of egg white into distinct fractions by chemical treatment and examined their effects on whip time and on the cake volume of angel cakes. Unfortunately it is not always clear which particular fraction was being used in the baking tests. Adjustment of egg white to pH 5 and removal of the resulting gelatinous precipitate (considered to be predominantly ovomucin but also containing some lysozyme) caused a decrease in viscosity and a marked loss of foam stability; both properties were largely restored by addition of the precipitate. Preparation of angel cakes with 'globulin- and ovomucin-free fraction' required a much longer whip time and resulted in a cake of much smaller volume than that prepared from untreated egg white. Replacement of the globulin fraction resulted in recovery of the normal cake volume but only partial recovery of whip time, and replacement of ovomucin decreased the whip time, but had no effect on cake volume. Extra ovomucin added to egg white markedly decreased the cake volume but addition of extra 'globulin-fraction' caused a decrease in whip time and increase in cake volume. It was found possible to make a cake with ovalbumin alone, but this needed a long whipping time to give sufficient foam and the cake had a coarse texture resulting from the lack of fine bubbles characteristic of the globulins.

The authors postulated the functions of the different protein fractions as follows: the globulins are largely responsible for good foaming and are particularly important in forming a batter with a large volume, small bubbles and a smooth texture; ovomucin is not a foamer but stabilizes egg white foams after a short whipping time, presumably on account of its rapid insolubilization at the bubble surface; other proteins (including

ovalbumin) furnish heat denaturable bulk which becomes insoluble during baking and forms the supporting matrix of the cake.

Forsythe & Bergquist (1951) found that blending egg white resulted in an initial rapid decrease in the fibre length of ovomucin, followed by a slow drop to a constant length of about 200 μ. Optimum foaming rates and cake volumes were obtained by decreasing the fibre length to 300–400 μ. When egg white was foamed the major proportion of the ovomucin was concentrated in the foam.

Studies on the change occurring in the functional properties of egg white as a result of contamination with yolk were reviewed and extended by Cunningham & Cotterill (1964). Addition of increasing amounts of yolk to a sample of white had only a small effect on surface tension and viscosity but caused regular decreases in foam volume, foam stability and angel cake volume. An interesting observation was that the optical density at 550 mμ increased linearly with increasing concentration of yolk. An improvement in angel cake volume was noted when the white was centrifuged to remove precipitate, particularly when the yolk contamination was low. The percentage of lipids in the precipitate from yolk-contaminated white was found to depend on pH value and to be affected in different ways by different chemical additives; the authors suggested that the precipitate contained a lipid–ovomucin complex.

Cotterill, Seideman & Funk (1965) later reported that the functional performance of yolk-contaminated egg white, as assessed by angel cake volume, could be improved by heat treatment, e.g. 15 min at 130°F, or by adjusting the pH to 6·5 or 10·0.

Effect of pasteurization

Moller-Madsen (1958) reported that liquid whole egg could be pasteurized in a coil type of milk pasteurizer at 158–159·8°F (70–71°C) for 40–180 sec without coagulating. When the temperature rose above 143·6–147·2°F (62–64°C) there was an increase in viscosity, but pasteurization up to 158°F (70°C) for 40 sec had no demonstrably deleterious effect on baking quality when the egg was used for making sponge cakes.

In 1952, Winter reviewed existing knowledge on the pasteurization of liquid whole egg and quoted the finding of Hanson, Lowe & Stewart (1947) that there was less than 5% decrease in volume of sponge cakes made from whole egg pasteurized at 141–144°F (60–62·2°C) for $1\frac{1}{2}$–$6\frac{1}{2}$ min or at 146–148°F (63·3–64·4°C) for 0·1–10·5 min, but that cakes made from the pasteurized egg were somewhat less desirable in texture and shape characteristics. However, Winter (1952) reported that whole egg pasteurized at 144°F (62·2°C) for as long as 30 min before freezing made as good custard, mayonnaise or sponge cake as unpasteurized frozen egg or the shell egg controls.

In a method of pasteurization of liquid whole egg employed in Northern Ireland (Anon., 1950) the egg was held at 142°F (61°C) for 3 min. It was stated that heat had no injurious effects on the whipping qualities of the egg and that the pasteurized egg showed a slight advantage over raw egg in baking tests; it was believed that this might have been due to more complete emulsification and more careful filtering.

The baking properties of egg, with special reference to pasteurized frozen whole egg, were comprehensively discussed by Farrand (1956). He stated that the pasteurized egg imported from the continent shortly after the war was not well received by the trade, probably for the following reasons: (1) the defrosted egg was very fluid and this created a false impression of its potential baking quality; (2) its composition was extremely variable, e.g. total solids content varied between 21 and 29%; (3) a number of samples were partially coagulated; and (4) occasionally the egg was musty. When a regular supply of frozen pasteurized egg prepared from sound shell eggs was provided for cake manufacture the baking properties were at least equal to those of imported frozen egg. The pre-pasteurization homogenizing caused the final product to have a low initial 'viscosity', but it was found that cold storage at freezing temperatures resulted in a gradual increase in viscosity and that this maturing period appeared to be of real practical importance with regard to baking characteristics. He went on to discuss methods of assessing the baking quality of frozen whole egg, and drew attention to two drawbacks of the type of aeration test generally used. Firstly, measurements dealing only with the quantity of air incorporated are quite inadequate to define aeration as manifest by the volume and crumb structure of the cake, as the size and variation in size of the individual air bubbles are also important parameters. Results from the conventional method of weighing a standard volume of mix may be expressed in different terms, but of these only 'percentage overrun', defined as the percentage increase in volume of the air free mix due to the incorporation of air, is linearly related to the amount of air in the mix. Secondly, standardization of any aeration test is very difficult because far too many variables are involved; the following factors are known to affect aeration: (1) quality and previous history of the egg, (2) particle size of the sugar, (3) ratio of egg to sugar, (4) proportion of sugar in solution at start of aeration, (5) size and type of machine, (6) speed of whisk, (7) time of mixing, (8) ingredient and ambient temperature, and (9) relative humidity of the atmosphere. Furthermore, some of these factors may interact, and if a pressure whisk is being used this adds further complications. For a performance test described in the paper, aeration to a standard percentage overrun, and not for a standard time, was chosen.

Among the factors involved in assessing the optimum baking conditions the following three merit special consideration: (1) rate of heat penetration in relation to the shape of the cake, (2) heat stability of the air bubbles, and (3) coagulation ('setting') temperature of the mix. The rate of heat penetration is not only uneven but is very sensitive to the amount of air in the mix. Heat stability of bubbles depends on their size and distribution. Setting of the cake depends mainly on the effect of the sugar–water ratio on coagulation of the egg, on the characteristics of the flour and the swelling of the starch. Consequently, use of a specially standardized flour is essential. Finally, accurate oven control is paramount.

In a second paper, Farrand (1957) reiterated some of the problems mentioned above and put forward a method for the qualitative measurement of aeration,

depending on the size-frequency distribution of air bubbles. A small sample of aerated mix is spread on a microscope slide and allowed to dry, and the diameters of the air bubbles are measured and counted, using a $\frac{1}{6}$-in. objective and a calibrated graticule. Mathematical treatment of the results leads to calculations of the average bubble size, the number of bubbles per gram of mix and the bubble surface per gram of mix.

An example was given of the application of this method to the aeration of a sponge sandwich batter. When equal weights of egg and sugar only were beaten at medium speed in a Hobart mixer the percentage overrun increased linearly with time up to about 280%, following which it first levelled off and then began to fall, after reaching a maximum of 330%. In the initial stages the air was incorporated in the mix mostly as very large bubbles. As aeration proceeded, both the average size and the variation in size of the bubbles were reduced, the number of bubbles increased rapidly and the total bubble surface increased relatively slowly. There was a marked difference when a mixture of egg, sugar and flour (1 : 1 : 0·6) was beaten. The percentage overrun quickly reached a maximum of just over 100%. Although bubble size decreased as aeration proceeded both the size and variation in size of the bubbles were much smaller and their number was much greater; the total bubble surface, however, was similar to that found for the egg–sugar mixture. In both cases, completing the mix by stirring in flour reduces the percentage overrun and results in a small decrease in the average bubble size and an increase in the number of bubbles. Farrand concluded that the amount of air in a mix is not necessarily a measure of the volume of the cake baked from it.

In assessing the cake-making properties of egg, factors other than the amount of air that can be incorporated have to be taken into account, e.g. those concerning stability of the mix, rate of heat penetration, etc.

Three series of baking tests with pasteurized egg were reported by Murdock *et al.* (1960). The first series showed that there was little difference in quality between sponge cakes made from English egg which had been frozen, thawed, pasteurized at 147°F for $2\frac{1}{2}$ min and re-frozen, and those made from unpasteurized material. Sponge sandwiches and Madeira cakes made with raw and pasteurized eggs from Denmark, China (pasteurized in England and Germany) and Northern Ireland were, with one exception, satisfactory. Use of pasteurized egg resulted in slightly lower cake volumes, although the differences were never greater than 10%, and also slightly lower foam volumes, but the ratings for grain, colour and crumb were the same for both types. The single exception was considered to be an unrepresentative sample. It was also noted that Polish egg, which is pasteurized at a high temperature, had been criticized by English bakers. In the third series of baking tests, sponge and Madeira cakes were made from liquid whole egg (from Northern Ireland) before and after pasteurization for 2 min 50 sec at different temperatures in the range 143–150°F (61·7–65·6°C). Foam volume was unaffected by pasteurization at 143°F, but decreased slightly (by less than 6%) when the egg was treated at 146°, 148° and 150°F. In all cases there were

slight reductions, but never more than 3%, in cake volumes but no differences in the ratings for grain, colour and crumb.

Views of cake manufacturers on the large scale use of pasteurized eggs were expressed at the Cake and Biscuit Alliance Technologists' Conference (Anon., 1961). Experiences of different manufacturers varied, some saying that results from pasteurized egg were not quite as good as those from frozen unpasteurized egg, whereas others stated that pasteurized egg made quite as good cakes as unpasteurized.

It was further commented that early results had not been very good because both egg and cake manufacturers had had little practice with the product, but more recent experience had shown that pasteurized egg produced very good results. It was thinner than frozen egg and needed a little longer whisking to beat it up to its full volume.

Heller *et al.* (1962) recorded results of baking tests on two batches of frozen whole egg, one unpasteurized and the other pasteurized at 148°F (64·4°C) for 2½ min (the present standard treatment). The latter had been homogenized and was visibly much 'thinner' than the raw sample; it should be noted that homogenization is not used in present practice. Nine bakeries compared these samples in various products, using the same processes in both cases. Two bakeries gave unfavourable reports, each on one product only, but in all other cases the products were rated as 'acceptable' and comments generally indicated that there was little or no difference between the two samples. The authors drew attention to the development in 1961 of an increasing demand from the trade for pasteurized whole egg.

Knight (1963) described the manufacture of pasteurized egg and discussed its performance in the bakery. He noted that there had been a reluctance by some bakers to use pasteurized egg and remarked that some of the early pasteurized products were of poor quality. In spite of lack of evidence, many bakers associated viscosity ('body') of egg with its performance in the bakery and earlier batches of pasteurized egg had a low viscosity, resulting from the largely discontinued practice of using high-pressure homogenization. More recently a number of bakeries had obtained consistently good results with pasteurized egg for a considerable time, but some difficulty had been experienced with certain products, notably sponges, choux paste and baked custards. Examples were given to illustrate how some of these difficulties with sponge cakes and eclairs could be overcome by suitably adjusting either the recipe or the method of manufacture.

Sugihara, Ijichi & Kline (1966) studied the effect of pasteurizing liquid whole egg for 3½ min at temperatures between 142° and 148°F (61·1–64·4°C) on *Salmonella* destruction, viscosity and baking performance. Viscosity was not affected unless the egg was pre-homogenized and frozen. Performance in layer cakes and commercial-type sponge cakes was unaffected by pasteurization, except for a single case at 148°F, when a 7% reduction in sponge cake volume was noted. Other baking tests, which also involved the effects of pre-homogenization and of variations in beating time, were carried out on 'true' sponge cakes made from a high egg (47%)–low flour (19%) batter.

Although homogenization and freezing of unpasteurized egg increased both foam and cake volumes, homogenized egg pasteurized at 148°F and frozen gave only 5% lower cake volume than the corresponding unhomogenized unpasteurized frozen egg; volume reductions were lower when lower pasteurization temperatures were used. In general, increasing the beating time resulted in parallel increases in foam and cake volumes, except for homogenized egg pasteurized at 148°F and frozen; this gave higher cake volumes than the control for beating times up to 200 sec and lower volumes for longer times. For unfrozen egg, foam and cake volumes were unaffected by pasteurization at 143° and 145°F unless the egg had been homogenized in which case the baking quality was adversely affected. When an extra flash-heating treatment (165°F for 2–3 sec) was included, there was an enhanced destruction of *Salmonellae* but no further effect on baking properties.

Experimental methods

Analysis

Total solids and fat were determined using the A.O.A.C. methods (1960) and protein by the Kjeldahl method using selenium and copper sulphate catalysts. Amylase activity was assessed by the method laid down in the Liquid Egg (Pasteurization) Regulations (1963) the starch substrate being prepared from a 'standard' soluble starch obtained from the British Egg Marketing Board.

Baking tests

Sponge cakes, choux paste and baked custards were made using the recipes and methods given below. The specific volume of egg/milk/sugar foams and of batters were measured using a calibrated scoop. Sponge volumes were determined about 18 hr after baking by measuring their height and the area of the surface made by cutting the sponges in half (Coppock & Cornford, 1960). Eclair volumes were measured by seed displacement and custard volumes assessed by measuring their height at the centre, the average height of five custards being reported.

All egg samples were carefully mixed before testing, separated samples being removed from the well-mixed egg for analytical examination. Frozen egg samples were defrosted overnight using running cold tap water. The temperature of all ingredients was adjusted to 70°F before use.

'All-in' sponge batters were mixed for 21 min in a Hobart cake mixer, the flour being whisked with the liquid ingredients. 'All-in' sponges containing emulsifier were mixed in a laboratory-scale Morton pressure whisk for 4·5 min at 15 lb/in^2 pressure. In the case of 'sponge batter' sponges the egg, milk and sugar were whisked for 10 min in a Hobart cake mixer before adding the flour.

The water and margarine were brought to the boil, the flour added and the mixture cooked for 2 min. After cooling to 120°F whilst beating at second speed, the egg was added slowly over 10 min the beating being continued over this period. After scraping

Sponge cakes

Ingredients	'All-in' sponges (oz)	'All-in' sponges (with emulsifier) (oz)	'$\frac{3}{4}$-egg' sponges (sponge batter method) (oz)
Whole egg	10	16	$7\frac{1}{2}$
Reconstituted skimmed milk	$3\frac{1}{8}$	$5\frac{1}{2}$	$2\frac{1}{2}$
Caster sugar	9	20	8
Chlorinated cake flour	8	20	8
Cream powder (sodium acid pyrophosphate type)	$\frac{1}{4}$	$\frac{1}{2}$	—
Sodium bicarbonate	$\frac{1}{8}$	$\frac{1}{4}$	—
Salt	$\frac{1}{16}$	—	—
Dibasic sodium phosphate	—	—	$\frac{1}{10}$
Water	—	$5\frac{3}{4}$	—
Proprietary monoglyceride emulsifier	—	$\frac{1}{2}$	—
Batter weight (oz)	5	7	5
Dimensions of baking tins (in.)	$5\frac{1}{2} \times 5\frac{1}{2} \times 1$	7 (diameter)	$5\frac{1}{2} \times 5\frac{1}{2} \times 1$
Baking time (min)	18	17	16
Baking temperature (°F)	390	410	410

Choux pastry

Ingredients	Weight (oz)
Water	10
Cake margarine	5
Strong flour	5
Egg	
Raw, frozen	11
Pasteurized, frozen	13
Pasteurized, chilled	8–11
Shell	7

Baked custards

Paste		Filling	
Ingredients	Weight (oz)	Ingredients	Weight (oz)
Soft flour	32	Fresh whole milk	10
Cake margarine	8	Whole egg	2
White fat	7	Sugar	1
Sugar	4		
Water	5		

down the bowl, the mixture was beaten for 30 sec at third speed and then piped out with a $\frac{5}{8}$-in. savoy tube on to a greased baking sheet. The eclairs were baked immediately at 450°F for 30 min.

The paste was made using a 'rubbing-in' method and shapes made by blocking on a machine using tins 3 in. in diameter at the top and $1\frac{3}{4}$ in. diameter at the bottom and a height of $1\frac{1}{2}$ in. The custard filling was prepared by mixing the egg, milk and sugar with a hand whisk. After allowing the mixture to stand for 1–2 min its appearance was noted and it was then re-mixed by hand whisking. The filling was scaled at $2\frac{1}{4}$ oz into pastry lined custard tins and immediately baked for 20 min at 430°F.

Results

Direct comparison of raw and pasteurized egg

In 1964 samples of frozen raw and pasteurized egg were received from twenty-eight processing plants eleven of which processed 'second quality' eggs. In each case both the raw and pasteurized samples were derived from the same bulk supply. The samples were examined after periods of up to 10 weeks after freezing, the majority being tested within 4 weeks of processing.

The analytical results obtained are summarized in Table 1, which includes corresponding data on commercial fresh egg reported by Brooks & Taylor (1955). One striking feature was the small difference in the total solids contents of the raw and pasteurized samples. This difference was statistically significant and corresponded with a difference in fat content, the raw samples in almost every case containing slightly more fat than the corresponding pasteurized samples. Since both the raw and pasteurized samples were obtained from the same bulk supply, it is not easy to explain why this difference should have arisen. It seems probable, however, that some loss of fat occurred in the case of the pasteurized samples since the fat/protein ratio of the raw samples corresponds more closely to that published for commercial fresh eggs (Brooks & Taylor, 1955). The sampling procedure adopted may have been such as to draw off pasteurized egg from bulk supplies in which some separation of fat had taken place. Consideration of the quantities of material involved shows that losses of this magnitude could not be accounted for as deposits on the plates of the pasteurization units.

TABLE 1. Average composition of raw and pasteurized egg

	Total solids (%)	Protein ($N \times 6{\cdot}68$) (%)	Fat (%)	Fat/protein ratio
Raw egg	26·0	13·6	11·4	0·84
Pasteurized egg	25·7	13·6	11·1	0·82
Commercial fresh egg (Brooks & Taylor, 1955)	26·05	13·63	11·55	0·85

The mean analytical results obtained on the samples submitted by the eleven plants processing 'seconds' did not differ significantly from those in Table 1.

The amylase activity of the pasteurized egg samples ranged from $4\frac{1}{2}$ to 7+ using the disc specified in the *Liquid Egg* (*Pasteurization*) *Regulations* (1963) the average activity being about $6\frac{1}{2}$. All the samples satisfied the test laid down in the Regulations.

The results obtained on sponge cakes made by the 'sponge batter' method are summarized in Table 2. The means given exclude results obtained on a few samples (six raw and one pasteurized) of abnormal composition.

The mean specific volumes of the foams and sponges obtained using pasteurized egg were 6·1 and 4·5%, respectively, lower than those obtained using raw egg, although in several cases the loss of performance exceeded 10%. In the case of three of the pasteurized samples the final sponges were considered to be slightly inferior in quality but just acceptable by commercial standards. A statistical examination of the results showed that for both foam and sponge specific volumes the difference between the raw and pasteurized samples was significant.

All the raw egg samples were satisfactory in baking quality. In terms of foaming capacity the raw egg was slightly more variable than the pasteurized product. Using the specific volume of sponges as a criterion, there was little difference in the variability of the raw and pasteurized samples.

The difference between first and second quality egg was not statistically significant, and no connection was found between amylase activity and baking quality.

TABLE 2. Performance of raw and pasteurized egg in sponge cakes

	Foam specific volume (ml/g)			Sponge specific volume (ml/g)		
	Raw	Pasteurized	Difference (%)	Raw	Pasteurized	Difference (%)
Mean	4·45	4·18	−6·1	4·48	4·28	−4·5
Standard deviation	0·333	0·216	—	0·210	0·234	—

There was no obvious correlation between the length of time the samples had been held in cold store before examination and their baking performance.

It is interesting to consider whether the slightly inferior performance of the pasteurized egg could be accounted for, at least in part, by its slight deficiency in fat, since two samples of very low fat content gave the lowest foam and sponge specific volumes. There was also a tendency for those samples of high fat content to give above average volumes, but the overall correlation between fat content and baking performance was low. In addition, the examination on other occasions of samples of raw and pasteurized

egg of normal fat content gave results in general agreement with those given in Table 2, the loss of performance resulting from pasteurization being about 5%. It seems unlikely, therefore, that the slightly lower fat content of the pasteurized egg was responsible for the small loss of performance found in the case of the samples under consideration.

Of the twenty-seven samples of pasteurized egg examined, eleven were somewhat different in appearance from normal raw egg, being slightly 'broken' or 'curdled', but there was no obvious correlation between the appearance of the pasteurized egg and its sponge baking performance, or between its appearance and the time it had been held in the frozen state after pasteurization.

In commercial practice, especially in large-scale work, an 'all-in' sponge is normally made, and the recipes and methods employed are such that reasonable variations in egg quality can be tolerated without adverse effects on the final product. Samples of chilled liquid raw and pasteurized egg subsequently received on four occasions from each of two processing plants were examined in an 'all-in' sponge recipe incorporating an emulsifier and the results obtained are summarized in Table 3.

TABLE 3. Performance of raw and pasteurized egg in 'all-in' sponges

	Sponge specific volume* (ml/g)		Difference (% of raw)
	Raw	Pasteurized	
Range	5·68–6·09	5·89–6·17	−3 to +6
Mean	5·97	6·01	+0·7

* By seed displacement.

On only one occasion was the difference between the raw and pasteurized samples significant, the raw egg being inferior. The difference in the mean results was not significant. These and results obtained on other occasions indicate that pasteurization is unlikely to have an adverse effect on the baking quality of egg when used in 'all-in' sponges.

The results obtained when twenty-three of the twenty-eight pairs of samples were used to make baked custards are summarized in Table 4. Using the height of the custards at the centre as a criterion, the pasteurized samples were slightly inferior to the corresponding raw egg samples, the mean difference being 3·9%. This difference is statistically significant. All the raw egg samples gave acceptable results whereas two of the pasteurized samples yielded unsatisfactory custards.

The main difficulty experienced with pasteurized egg in this series of tests was the tendency for a thick yellow skin to form on the surface of the custard and for the surface to crack. In addition, few of the samples gave rise to a brown 'penny', a feature often considered to be important.

TABLE 4. Performance of raw and pasteurized egg in baked custards

	Height of custards at centre (mm)		
	Raw	Pasteurized	Difference (% of raw)
Range	16·4–18·4	15·2–17·6	−11 to 0
Mean	17·3	16·6	−3·9

In contrast to the experience with sponges, the appearance of the defrosted pasteurized egg appeared to be a rough indication of its performance in baked custards. Samples with a 'broken' or 'curdled' appearance invariably produced slightly inferior products, whereas rather less difficulty was experienced with samples of normal appearance.

The effect of cold storage on the baking quality of frozen pasteurized egg

Samples of raw and pasteurized egg were obtained from each of three U.K. processing plants. All three plants processed first quality eggs, and in each case the raw and pasteurized samples were derived from the same bulk supply of freshly broken-out shell egg. The egg samples were frozen in 7-lb tins and stored in a local cold store at 12–16°F. At approximately monthly intervals samples were withdrawn from the cold store and examined for baking performance in sponges made by the 'sponge batter' method, baked custards and choux paste. After about 1 year the interval between examinations was increased to approximately 2 months.

The analytical characteristics of the samples are given in Table 5. Pasteurized egg received from Plants A and B contained slightly less fat than the corresponding raw samples and both the raw and pasteurized egg from Plant C contained slightly more fat than usual. All three pasteurized samples satisfied the statutory amylase test.

The individual results obtained in 'sponge batter' baking tests are shown in Figs. 1 and 2. The changes in foam and sponge specific volumes, expressed as percentages of the initial values, are given in Figs. 3 and 4, respectively. In initial performance, the three pairs of samples were normal, the differences between the raw and pasteurized samples being comparable to those found earlier in the case of the samples received from most of the U.K. processing plants.

The behaviour of the individual samples differed somewhat during the course of the experiment. In terms of foam specific volume, both the raw and pasteurized egg from Plant A steadily improved during the first 20 weeks of storage, whereas the egg from Plants B and C showed little change in performance. This difference in behaviour may be associated with the relatively inferior initial performance of the egg from Plant A since after about 20 weeks storage the foaming ability of all three raw egg samples was similar and this applied also to the three pasteurized samples. In the case of the raw

TABLE 5. Analytical characteristics of raw and pasteurized egg used in the experiments to determine the effect of cold storage on baking quality

Processing plant		Total solids (%)	Protein ($N \times 6{\cdot}68$) (%)	Fat (%)	Amylase disc No.
A	Raw	26·0	13·9	11·1	—
	Pasteurized	25·7	13·9	10·8	$6\frac{1}{2}$
B	Raw	25·8	13·6	11·5	—
	Pasteurized	25·7	13·6	11·1	$6\frac{1}{2}$
C	Raw	26·0	13·7	11·8	—
	Pasteurized	26·3	13·6	11·7	$5\frac{1}{2}$

egg, little change in foam specific volume occurred after about 20 weeks storage. The three pasteurized samples deteriorated in foaming ability after about 20 weeks storage, this deterioration being most marked with the samples from Plants A and C. Both these samples, particularly the egg from Plant C, improved in performance after about 50–60 weeks storage and then deteriorated again after about 80 weeks storage.

In terms of sponge specific volume, all three raw samples improved during the first 40 weeks of storage and apart from sample A which deteriorated slightly after about 60 weeks, changed little thereafter. The pasteurized samples, particularly B and C,

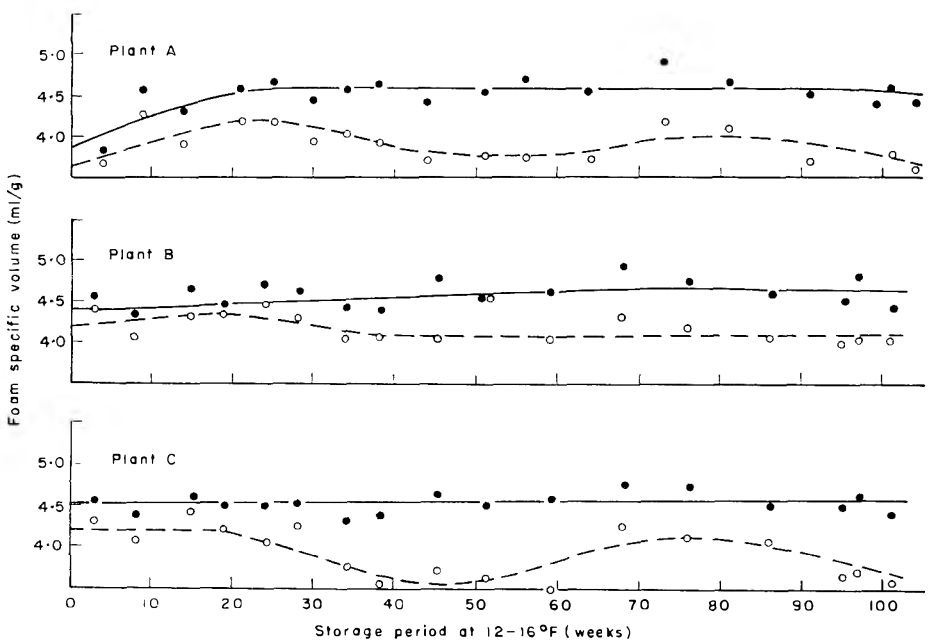

FIG. 1. Effect of cold storage on foaming ability of raw (●) and pasteurized (○) egg.

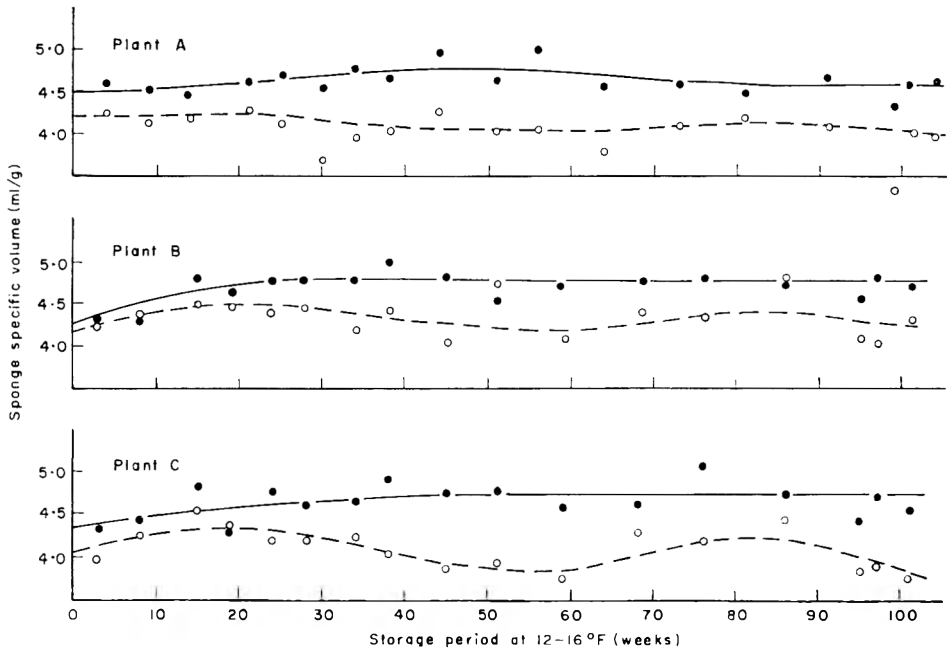

FIG. 2. Effect of cold storage on baking quality of raw (●) and pasteurized (○) egg.

improved during the first 20 weeks of storage but then deteriorated. After about 60 weeks all three samples improved in baking quality but this improvement was not sustained, all three samples again deteriorating after about 80 weeks storage.

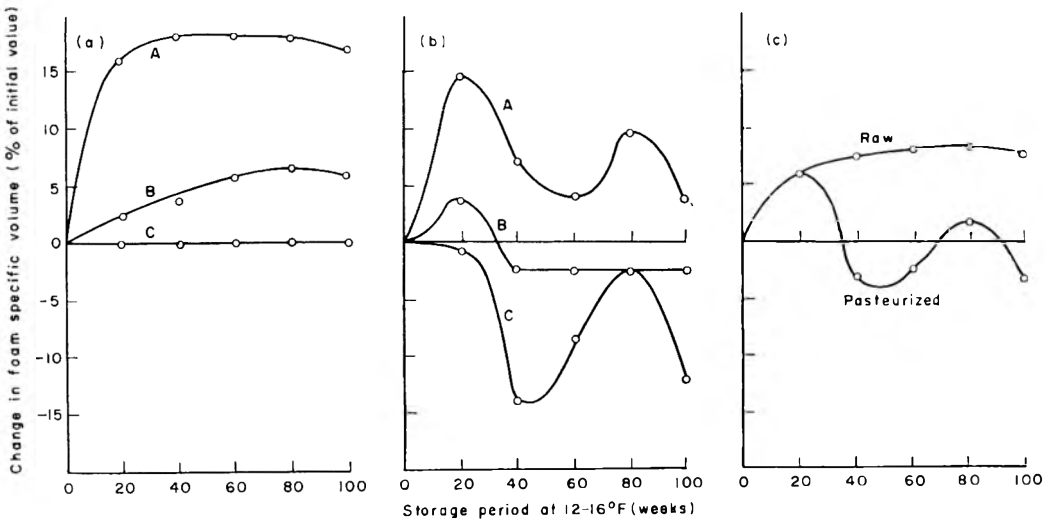

FIG. 3. Change in foaming ability of raw (a) and pasteurized (b) egg during cold storage. (c) Mean performance.

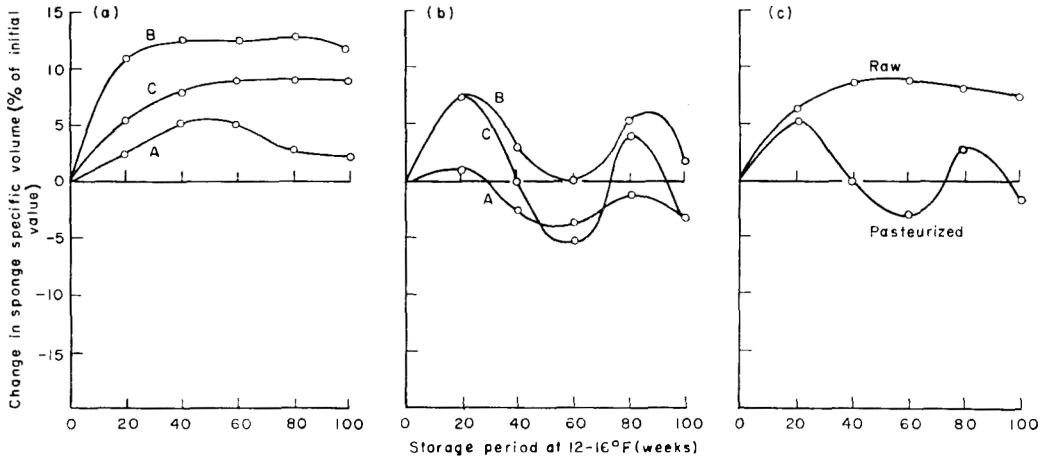

FIG. 4. Change in baking quality of raw (a) and pasteurized (b) egg during cold storage. (c) Mean performance.

The relative performance of the three pairs of samples is given in Fig. 5. It will be seen that in all cases the pasteurized egg suffered in comparison with the corresponding raw samples throughout the duration of the experiment, and that the mean differences in both foam and sponge specific volumes followed the same pattern. Little or no loss in the relative quality of the pasteurized egg was observed during the first 20 weeks

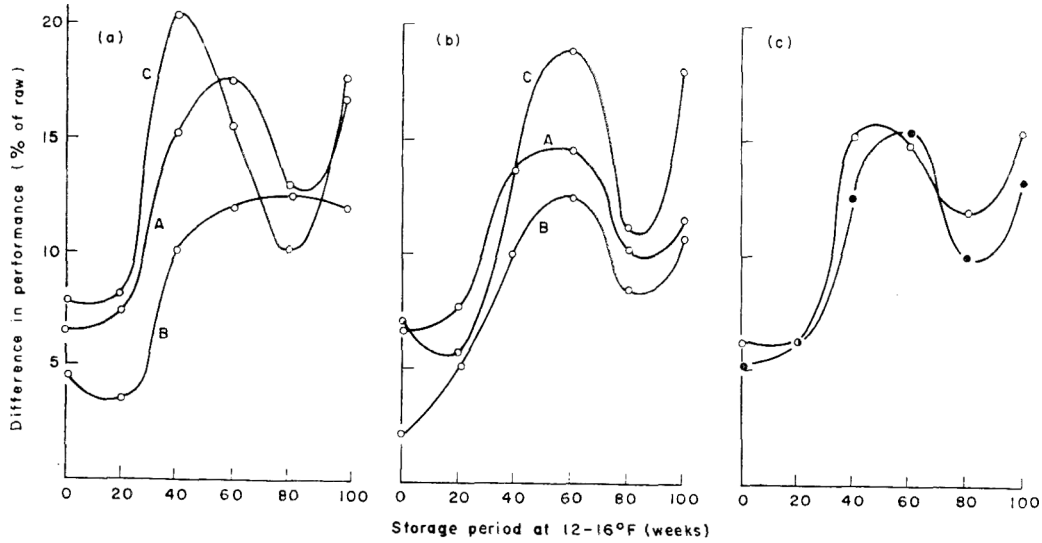

FIG. 5. Relative performance of raw and pasteurized egg during cold storage. (a) Foam specific volume; (b) sponge specific volume; (c) mean difference in performance: ○, foam; ●, sponge.

storage but a quite rapid and marked deterioration occurred between 20 and 40 weeks storage. After about 50 weeks this deterioration was arrested and the relative performance of the pasteurized egg then showed some improvement. However, the pasteurized samples deteriorated again after about 80 weeks.

These results suggest that after about 20 weeks in cold store frozen pasteurized egg is likely to deteriorate in baking quality and that after about 40 weeks its performance will be approximately the same as that of freshly frozen pasteurized egg and about 15% lower than that of raw egg stored for the same time under the same conditions. The most marked deterioration in the baking quality of the pasteurized egg used in this investigation occurred between about 20 and 40 weeks storage and this suggests that it may be undesirable to store pasteurized egg in the frozen state for such long periods.

The samples were not regularly examined for their performance in 'all-in' sponges, this type of recipe being less sensitive to changes in egg quality than 'sponge batter' sponges. However, after approximately 2 years storage the three pairs of samples were used to make 'all-in' sponges and high ratio fruit cakes and the results obtained are given in Table 6. All samples were judged satisfactory.

TABLE 6. Performance of 'old' raw and pasteurized egg in 'all-in' sponges and high ratio fruit cake

	Plant A		Plant B		Plant C	
	Raw	Pasteurized	Raw	Pasteurized	Raw	Pasteurized
All-in sponge						
Specific volume (ml/g)	6·03	5·73	5·76	5·83	5·88	5·74
High ratio cake						
Specific volume (ml/g)	2·11	2·08	2·10	2·09	2·09	2·07
% fruit in top half of cake	48	54	46	48	49	48

In initial performance in baked custards the three pairs of samples were normal, the raw egg being slightly superior to the pasteurized egg, the use of which gave rise to custards lacking a surface 'penny'. In terms of custard height, the slight difference in favour of the raw egg was maintained by the egg received from Plants A and B during the course of the experiment. In the case of the egg from Plant C the gap between the raw and pasteurized egg increased slightly after about 40 weeks cold storage.

The quality of the custards made with pasteurized egg deteriorated as the age of the egg increased, the deterioration being associated with surface cracking and a lack of stability. After about 10 weeks storage two of the three pasteurized samples gave rather unsatisfactory results and the third performed poorly after about 30 weeks, whereas good quality custards were obtained with the raw egg even after about 100 weeks cold

storage. Judging from these and other results it would be expected that commercially unsatisfactory baked custards would most probably be obtained if frozen pasteurized egg more than about 30 weeks old were used, the deterioration observed being paralleled by a loss of baking quality in 'sponge batter' sponges. It should be noted that homogenization of the egg or of the custard mix or amendments to the recipe or method will enable much more satisfactory results to be obtained.

In choux paste the raw samples initially gave slightly better overall results than the corresponding pasteurized samples. As the age of the egg increased there was an increasing tendency for the pasteurized egg to deteriorate, this being fairly evident after about 30 weeks storage. The raw egg gave good results in choux paste even after 100 weeks storage.

The separation of chilled pasteurized egg during transit

It is known that separation tends to occur when liquid pasteurized egg is allowed to stand without agitation, the top layer of the egg becoming rich in fat. In order to assess the extent to which this separation is likely to occur under commercial conditions and the effect such separation may have on baking quality, a sample of chilled liquid pasteurized egg which had been transported in an egg tanker lorry was compared with a control sample taken before transit. It was found that the transported sample did not have as good whipping properties as the control and gave rise to sponges of lower specific volumes. The transported sample, which was obtained from the bottom of the egg tank, contained less total solids (24·8%) than the control (25·7%) indicating that some separation had occurred.

In a subsequent experiment samples of chilled liquid pasteurized egg taken before and after a journey of 200 miles in a full egg tanker were examined. The samples taken after transit were drawn from the top and bottom of the tanker.

The sample taken from the bottom of the tanker was relatively low in fat and high in protein and had poorer whipping properties than the other samples which were similar in this respect. The sponges and baked custards made with this sample were also slightly inferior to those made with the other samples. All four samples performed satisfactorily in choux paste.

These results clearly showed that some separation of fat can take place during the transit of liquid pasteurized egg in a full tanker and indicated that if separation does occur the egg at the bottom of the vessel will be slightly inferior in baking quality to the rest of the consignment. In order to determine the effect of transporting egg in a partly filled tanker a further experiment was carried out in which a two-compartment tanker was used, the front compartment being full of pasteurized egg (1000 gal) and the rear compartment half-full. The tanker was driven approximately 200 miles over a period of about 10 hr and at the end of the journey the egg in the top and bottom of each compartment was sampled, about 2 gal of egg being discarded before the bottom samples were taken. Samples were also taken from each compartment after 'plunging'

with a sterile milk plunger for several minutes. The egg was also sampled at the time the tanker was loaded.

No separation of fat occurred in the half-full compartment, the movement of the tanker being sufficient to keep the egg well mixed. In the full compartment the top layer contained significantly more fat than the bottom, but the separation of fat was less marked than in the previous experiment.

The sponge-making quality of the sample drawn from the bottom of the full compartment was not as good as that of the other samples, as it gave sponges of slightly smaller volume. All samples gave similar results in baked custards and choux paste.

The effect on baking quality of 'ageing' chilled pasteurized egg

In the experience of certain large-scale bakeries, liquid pasteurized egg improves in baking quality when kept for several days at 35–40°F before use. It was considered desirable to carry out carefully controlled tests to obtain further evidence on this point.

In the first, a sample of chilled pasteurized egg was received within a few hours of pasteurization and duplicate baking tests were carried out on each of five successive days, the egg being tested in 'sponge batter' sponge cakes. Throughout the storage period the egg was maintained at a temperature of 38–40°F without agitation except immediately prior to samples being withdrawn for the baking tests.

In the second experiment a similar procedure was adopted except that on each day duplicate baking tests were carried out using each of two different ovens.

The differences in performance of the egg samples during the periods of the experiments were not significant. This applied to both foam and sponge specific volumes. Since the 'sponge batter' test is sensitive to changes in egg quality it may be concluded that, at least in small-scale baking, the performance of liquid pasteurized egg is unlikely to change over a period of 4 days.

The effect of pasteurization temperature on baking quality

The statutory pasteurization conditions (heating at not less than 148°F for a minimum time of 2·5 min) were chosen not only because they are adequate to destroy *Salmonellae* but also because virtually all the amylase activity of egg is destroyed (Shrimpton *et al.*, 1962), and by adopting this combination of temperature and time an amylase test to confirm adequate pasteurization could be included in the Regulations.

Although experience obtained before this legislation was introduced had indicated that the conditions of heating subsequently adopted were not likely to lead to difficulties associated with the baking quality of pasteurized egg (Heller *et al.*, 1962), it is well-known that excessive heat treatment of egg leads to deleterious effects on baking quality.

In order to assess the effect of temperatures of pasteurization slightly below that laid down in the Regulations, samples of egg were pasteurized in a commercial plant at 145°, 146°, 147° and 148°F. The plant was set to run at 148°F and then adjusted to reduce the temperature at the outlet of the holding section to 145°F in successive steps.

Samples were taken at the appropriate temperatures and examined for baking quality, using a sample of raw egg taken from the float chamber as a control. The results of the 'sponge batter' sponge tests, together with the amylase activity of the samples, are given in Table 7.

TABLE 7. Baking quality of egg pasteurized at temperatures ranging from 145° to 148°F

Sample	Foam specific volume (ml/g)	Sponge specific volume (ml/g)	Amylase disc No.
Raw	3·82	3·99	—
Pasteurized			
145°F	3·94	4·00	2
146°F	3·18	4·07	5
147°F	3·29	3·83	6½
148°F	3·37	3·86	7+

It is noteworthy that all the samples with the exception of the one heated at 145°F passed the statutory amylase test and it is clear that pasteurization temperature of 148°F need not be reached in order to ensure that the test is satisfied.

The foaming ability of the egg heated at 146°F and above was significantly lower than that of the raw egg and of the egg heated at 145°F. However, the effect of pasteurization temperature on sponge volume was small and probably not of commercial significance. All the samples performed satisfactorily in baked custards although the egg heated at 148°F gave rise to custards showing a greater tendency to 'weep'. Consistently satisfactory results were obtained in choux paste. The overall effect of increasing the pasteurization temperature from 145° to 148°F was not marked but the baking quality of the egg processed at the lower temperature was judged to be higher than that heated at 148°F.

On another occasion samples of egg were commercially pasteurized at 149°, 150° and 152°F. The results obtained in the 'sponge batter' sponge test are given in Table 8.

TABLE 8. Baking quality of egg pasteurized at 149–152°F

Pasteurization temperature (°F)	Foam specific volume (ml/g)	Sponge specific volume (ml/g)
149	4·34	4·58
150	4·23	4·49
152	4·14	4·30

There was a small but definite loss of performance as the pasteurization temperature was raised from 149° to 152°F; a similar pattern was evident in the case of baked custards. Fairly satisfactory results were obtained with all samples in choux paste

although the egg processed at the lowest temperature was considered to be slightly inferior to the others.

It may be concluded that the use of pasteurization temperatures only a few degrees in excess of 148°F is likely to result in some loss of baking quality over and above that resulting from normal pasteurization. Judging from the small number of samples examined, this additional loss of quality is quite small (about 6% in terms of sponge volume) when the temperature is raised from 149° to 152°F and is comparable in extent to the loss of quality resulting from normal pasteurization.

The effect on baking quality of homogenizing frozen pasteurized egg after thawing

It has been observed that homogenization of defrosted pasteurized egg in a small hand-operated cream homogenizer may have beneficial effects on its baking quality especially if its performance when defrosted and handled normally is well below average. A similar improvement has resulted when poor quality pasteurized egg has been thawed mechanically, the effect on the egg of the fairly intense mechanical action involved presumably being comparable to that of homogenization.

The improvement resulting from these two processes is illustrated by Fig. 6 from

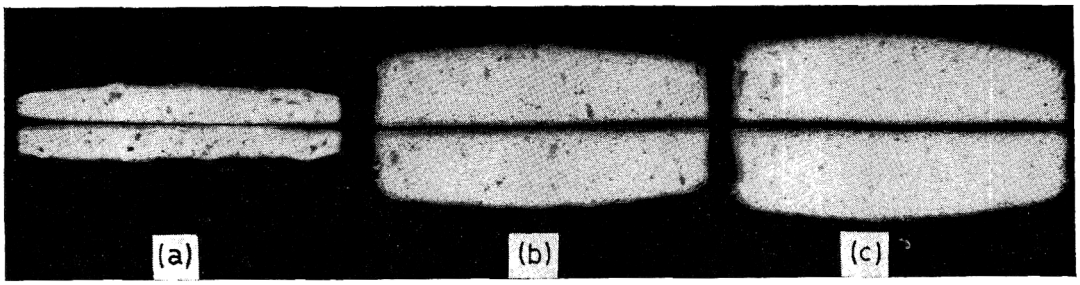

FIG. 6. 'Sponge batter' sponges made with: (a) frozen pasteurized egg, (b) the same egg mechanically defrosted, and (c) the same egg homogenized after normal defrosting.

which it can be seen that both mechanical thawing and homogenization of naturally thawed egg can give rise to a marked increase in baking quality. It should be noted that this sample of pasteurized egg had been held frozen for about 1 year and that it had been thawed and re-frozen before examination. The thawing and re-freezing processes certainly caused some deterioration of the egg which was, however, of poor quality when first thawed.

The beneficial effects of homogenization are also illustrated by Figs. 7 and 8 which show 'sponge batter' sponges and custards made from egg which had been held frozen for about 2 years before examination.

The improvement in baking quality brought about by homogenizing the egg was maintained when the egg was subsequently stored at 38–40°F for 4 days. Some improvement was also observed when homogenized egg was used in 'all-in' sponges. It should

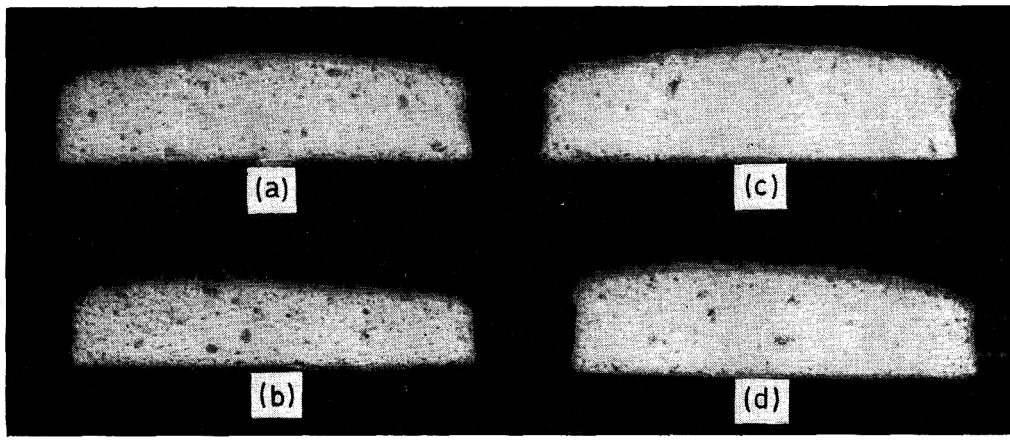

FIG. 7. 'Sponge batter' sponges made with frozen pasteurized egg: (a) and (b) defrosted normally, (c) and (d) homogenized after defrosting.

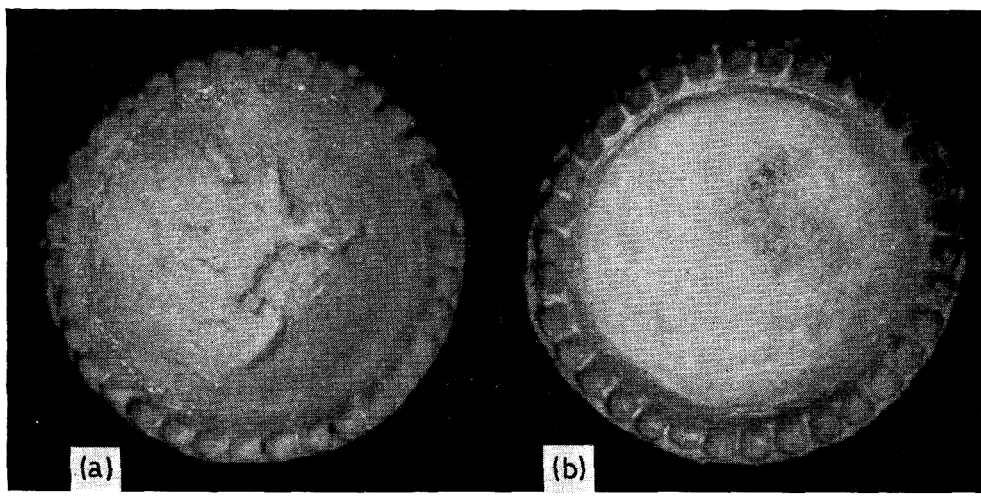

FIG. 8. Baked custards made with frozen pasteurized egg: (a) defrosted normally, and (b) homogenized after defrosting.

be noted that egg which performs poorly in 'sponge batter' sponges often shows little deterioration in 'all-in' sponges.

One of the main features of pasteurized egg which has been stored in the frozen state for long periods is its appearance when thawed, the egg tending to be 'broken' or 'separated'. The process of homogenization (or of mechanical thawing) markedly improves the appearance of such egg, there being an increase in smoothness. Experience suggests that a reversible aggregation of one or more egg components takes place during storage and that the application of shearing forces during homogenization or mechanical

thawing breaks down the aggregates so formed and gives rise to an improvement both in appearance and in baking quality. It is probable that this aggregation causes a breakdown in the oil/water emulsion with the consequent liberation of oil which has an adverse effect on baking quality, particularly in 'sponge batter' sponges and in custards. The deleterious effect of oil in the former and the separation of a yellow, oily layer on the surface of custards support this view.

Obtaining the best results with pasteurized egg

Although the average performance of pasteurized egg is only slightly inferior to that of raw egg, occasional samples are rather poor in baking quality. Particular attention has been paid to the conditions under which pasteurized egg will give the best possible performance and the results are summarized below.

Sponges. The main problems with pasteurized egg occur when the methods of sponge production used require the egg to have good foaming properties. It has been found, that extending the whisking time of pasteurized egg usually increases both foam and sponge specific volumes.

Pasteurized egg has been found to give better results in sponges when half the sugar is whisked with the egg and milk until a good foam is obtained, and the remainder of the sugar is then whisked in before blending in the flour. It has also been found that the addition at the whisking stage of about 12½% of the flour used in the recipe is beneficial. As noted earlier, an improvement in the performance of frozen pasteurized egg has also been observed when the egg has been homogenized before use. This effect is most marked in the case of egg of poor baking quality.

Baked custards. An increase in the proportion of egg in the receipe is often beneficial, and in the case of frozen pasteurized egg homogenization of the egg or the custard mix usually gives improved results. Warming the milk to 160°F before use and the addition of a small proportion (about 1% of the filling) of butter or margarine is helpful in aiding the formation of a 'penny'. The fat should be warmed with the milk to about 160°F.

Choux paste. The recipe and method used have an important effect on the quality of choux paste and best results have been obtained when the quantity of margarine in the recipe has equalled that of the flour. The amount of egg required has been found to vary depending on the type of egg used. In our experience and using the test recipe given under 'Experimental methods', the following quantities of egg are normally needed for 100 parts by weight of roux:

Shell egg	35–45 parts by weight
Frozen raw egg	55 parts by weight
Frozen pasteurized egg	65 parts by weight
Chilled liquid pasteurized egg	40–55 parts by weight

Adequate beating should be given during addition of the egg, under-beating rather than over-beating being sometimes responsible for poor results.

Conclusions

Pasteurization resulted in a small loss of baking quality in particularly sensitive products such as sponge cakes and egg custards. This was overcome by using increased whipping times, modifying the recipe and modifying the method of preparation. Storage of pasteurized egg in the frozen state caused a deterioration of products sensitive to egg quality after about 30 weeks, but the frozen egg gave satisfactory results in other baked goods after 2 years storage. The baking properties of thawed frozen egg were improved by homogenization.

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The influence of lipids on fish quality

R. G. ACKMAN

Summary. The lipids in fish muscle can influence product quality through interaction with other components. In frozen storage of lean fish such as cod and haddock it is established that hydrolysis of phospholipids gives free fatty acids capable of interacting with protein to produce texture deterioration, although the mechanism of this interaction is not yet clarified. Rancidity is more commonly associated with lipids in fatty fish. Some seasonal aspects of the rancidity problem for lean fish are discussed and it is suggested that some fish may be highly susceptible on a seasonal basis not through variations in the lipid or in the natural anti-oxidant tocopherol, but through the presence of other materials or biological processes predisposing tocopherol to rapid destruction, with consequent abnormal development of rancidity.

Introduction

Fisheries research, in common with other lipid research areas, has benefited enormously from the introduction of modern chromatographic techniques. The classic work on marine fats of J. A. Lovern, T. P. Hilditch and others (cf. Lovern, 1962, 1964) has grown into masses of detailed information on all types of marine lipids unimagined a score of years ago. One development which has had a simplifying effect on marine lipid research was the gradual realization that fish fatty acids, contrary to earlier speculation and conflicting evidence, conformed to normal biochemical patterns in that the double bond systems were methylene interrupted in the polyunsaturated acids, and the terminal double bond structures belonged principally to the oleic, linoleic or linolenic systems (Ackman, 1964). There are also structural similarities in the mono-unsaturated fatty acids (Ackman & Castell, 1966) and in the saturated fatty acids (Ackman & Sipos, 1965). Comparative studies (Gruger, Nelson & Stansby, 1964; Klenk & Eberhagen, 1962; Ito & Fukuzumi, 1963) have established that the lipids of commercially important species have essentially similar fatty acid composition patterns, with variations in eight or ten key components [myristic, palmitic, palmitoleic, stearic, oleic, eicosenoic, eicosatetraenoic (arachidonic), eicosapentaenoic, docosenoic and docosahexaenoic acids] accounting for the long known differences in properties such as iodine value.

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This is illustrated in Table 1, where it will be noted that these particular fatty acids account for over 90% of the totals in both herring oil (a depot fat) and in cod lipids (essentially phospholipids).

TABLE 1. Percentage composition of the major fatty acids in herring oil (a typical fish depot fat), in cod flesh (essentially phospholipids) and in the free fatty acids liberated from phospholipids in frozen storage

Fatty acid	Herring oil*	Fresh cod flesh lipids		Cod flesh FFA after 26 weeks frozen storage‡
		Nova Scotia†	North Sea‡	
14:0	6·4	0·4	0·5	0·7
16:0	12·7	24·1	20·6	26·3
16:1	8·8	1·1	1·6	1·6
18:0	1·0	3·4	4·2	3·6
18:1	12·7	7·8	10·1	10·0
20:1	14·1	1·2	1·5	1·4
20:4	0·3	3·5	2·9	2·2
20:5	8·4	17·6	14·6	16·5
22:1	20·8	1·1	Trace	Trace
22:6	4·9	37·5	35·4	34·4
Total % these components	90·1	97·7	91·4	96·7
Calculated (gross) iodine value	126	261	255	246

* Ackman & Eaton (1966).
† Ackman & Burgher (1964).
‡ Olley & Duncan (1965).

Despite the increased knowledge in these areas the subtle interactions of lipids with other materials of biological importance are only partly explored, although these interactions may be more important than the character of the lipids themselves and their alterations. The current status of the occurrence of some lipids in particular fish, and two aspects of the influence of lipids on fish quality form the basis of this report Earlier work in this respect has been summarized by Lovern (1962).

Lipids in fish muscle with particular reference to cod

It is frequently convenient to divide fish into rough classifications, in terms of muscle lipid content, for discussion of the properties of commercially valuable products such as

fresh or frozen fillets. There are important commercial species such as cod* and haddock which are definitely lean (< 1% lipid). In these fish the depot fats are stored in the liver. At the other extreme are the teleost fatty fish such as herring, mackerel, etc. In these fatty species the depot fats are chiefly stored in muscle tissue and also to a minor extent in mesenteric fat. Consequently the flesh shows marked variations in fat content with season and degree of sexual maturity. Herring, for example, can vary from 4 to 20% fat in the whole fish, with only slightly less variation in the muscle fat content (Lovern, 1938; cf. Wood, 1957); Iles & Wood, 1965). In between these extremes are many other commercially important species and some, such as halibut, sole and related species, can approach (≤ 1% lipid) the low level of the lean fish or may seasonally be somewhat richer in fat.

The distribution of depot fats in the muscle of fish varies widely with both species and type of muscle as illustrated by recent histochemical studies (Yamada & Nakamura, 1964), and the anatomical studies of Brandes & Dietrich (1958) and George & Bokdawala (1964). In any discussion of fish lipids it is, therefore, often very necessary that the part of the muscle involved be specified. This is true of the 'lean' species as well as the obviously fatty species. Thus the belly flap tissue in cod and haddock is slightly richer in lipid than the rest of the same muscle (Fraser, Mannan & Dyer, 1961). In cod, the tail section of whole fillets is slightly richer in lipid than the centre or head section (Dambergs, 1963). This is due to a surface band of darker muscle which forms a proportionately greater part of the tail flesh than of the rest of the fillet in cod and haddock (Fraser *et al.*, 1961; Castell & MacLean, 1964). In many experiments with cod the potential effects of this muscle layer have been disregarded, although in species where it is more obvious it is usually considered separately under the descriptive term red or brown lateral line muscle (Braekkan, 1956; Fraser *et al.*, 1961; Jonas & Bilinski, 1964). In cod, as in other species, the dark muscle is richer in fat, with a lipid content of 1·8–2·2% as against a normal range of 0·60–0·75% in the white muscle or whole fillet (Castell & MacLean, 1964; Bligh & Scott, 1966; Ackman & Cormier, 1967). It should be noted that these figures are based on the efficient extraction procedure of Bligh & Dyer (1959) and are therefore comparable.

The subject of the lipid content of the flesh of lean fish must be briefly mentioned since virtually all older data in reference books and also some quite recent publications are based on extraction procedures which give figures of the order of 0·2–0·3% lipid, which in the light of present knowledge is far too low. The isopropanol procedure of Dambergs (1963) gives results of about 1% lipid for cod fillets. The latter procedure

* Fish are referred to by common names which vary in usage in different marine areas. Those mentioned in this report are specifically: cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), herring (*Clupea harengus*), mackerel (*Scomber scombrus*), dogfish (*Squalus acanthias*), halibut (*Hippoglossus hippoglossus*), lemon sole (*Microstomus kitt*), petrale sole (*Eopsetta jordani*), commercial sole (largely blackbacked flounder, *Pseudopleuronectes americanus*), carp (probably *Cyprinus carpio*), sablefish (*Anaplopoma fimbria*), and rock cod (genus *Sebastodes*).

evidently includes as lipid some additional material, possibly intact lipoproteins, which could be subject to splitting in alternative procedures. Lovern (1965) has reviewed the applicability of the miscible chloroform–methanol–water system of Bligh & Dyer (1959) to fish flesh and other materials. An alternative chloroform–methanol procedure applied to North Sea cod gave an average lipid content of 0·67% (Olley, Pirie & Watson, 1962).

The seasonal variations in the extractable lipid of cod flesh are reportedly very slight (Lovern, Olley & Watson, 1959; Olley & Lovern, 1960; Dambergs, 1964; Jangaard *et al.*, 1967a). It should be noted that the Nova Scotia fish have been of medium size with few showing evidence of sexual maturity, although these few conform to data for the rest in percentage lipid. One lot of very small North Sea cod (*ca.* 1 lb) has been reported to have a slightly higher than normal lipid content in the flesh (Olley *et al.*, 1962). The fatty acids from the lipids of North Sea and Nova Scotia cod (Table 1) show a striking resemblance (Ackman & Burgher, 1964), although it has been observed that in very large cod deviations from the typical fatty acid composition sometimes occur (Jangaard, Ackman & Sipos, 1967b). The overall lipid composition of North Sea and Nova Scotia cod is probably also similar as indicated by the comparable rates of lipid hydrolysis during frozen storage (Lovern & Olley, 1962; Bligh & Scott, 1966). In addition, Love (1958) found that in severely starved North Sea cod the water content of muscle rose from a normal 80% to about 86%. Non-lipid solids must then decline approximately from 20 to 14%. Proportionately, cellular lipid would fall from the typical value of 0·76% to 0·53%, and in fact lipid was found to be 0·54% in muscle of a severely starved Nova Scotia cod (Ackman & Cormier, 1967).

There is a heavy fishery for cod in summer off the coast of Newfoundland referred to as the trap fishery since the fish come inshore in pursuit of capelin and are taken in trap nets. These fish are feeding very heavily and it has been observed that the muscle lipid averages 0·91% as compared to an average of 0·63% for Newfoundland offshore trawl-caught fish (MacCallum *et al.*, 1964). There is no significant difference in the types of lipids found in trap cod (Bligh, private communication). A few cod taken from Nova Scotia waters in July 1966, were found to average 0·83% lipid by the method of Bligh & Dyer (1959) (Ackman & Cormier, unpublished results), whereas the same operator consistently obtained a typical figure of 0·75% in winter months (Ackman & Cormier, 1967). The July maximum by this extraction technique in this particular case (cf. Jangaard *et al.*, 1967a) is thus comparable to the Newfoundland trap cod results and those reported by Dambergs (1964).

In most commercial size cod the extractable muscle lipids must consist almost exclusively of cellular lipids since the concentration of these lipids in the tissue is not known to fluctuate as markedly as do depot lipids. There is in fact a close resemblance of the fatty acid compositions of cod lipids with the fatty acids of the phospholipids of lemon sole and halibut (Olley & Duncan, 1965), and with the fatty acids of mitochondria from heart muscle of fish such as rock cod, cod and petrale sole (Richardson

et al., 1962). The dark muscle in cod, although richer in extractable lipid than the white muscle, has essentially the same proportions of the same types of lipid (Bligh & Scott, 1966). This observation is important in that most experiments on cod muscle have been based on whole fillets, possibly containing a varying proportion of the dark muscle. The effect of the latter on the results can, therefore, be considered to be slight in most cases.

Lipids and quality in frozen cod

The cod is, for the above reasons, a less complicated fish to work with experimentally from the lipid point of view than fatty or semi-lean species. This is fortunate in view of the commercial importance of cod in North Atlantic fisheries. In the fatty species of fish it is accepted that in commercial handling of either fresh or frozen material there will inevitably be development of rancidity (Banks & Hardy, 1965). In unfrozen cod serious bacterial spoilage has, in the past, usually preceded development of rancidity, although with the advent of modern handling procedures and treatment with antibiotics or irradiation, rancidity may again become a limiting factor in the life of this product. In frozen storage of cod rancidity is seldom a serious factor in keeping quality. The problem in frozen storage of cod is normally one of texture deterioration, indicating denaturation of protein (Dyer, 1951; Ironside & Love, 1958; Love & Ironside, 1958), and such denaturation may be correlated with the reduced extractability of the protein component actomyosin with 5% sodium chloride solution (Dyer, French & Snow, 1950; Love, 1958).

The development of free fatty acids (FFA) in stored frozen fish has been recognized as occurring concurrently with protein denaturation, possibly prior to the latter process, and undoubtedly related to it (Dyer & Morton, 1956; Dyer & Fraser, 1959; Olley & Lovern, 1960; Olley *et al.*, 1962). In an attempt to find a basis for the relationship postulated between FFA formation and protein insolubility a series of studies were carried out in one laboratory (King, Anderson & Steinberg, 1962; Anderson, King & Steinberg, 1963; Anderson & Steinberg, 1964; Anderson, Steinberg & King, 1965). The addition of fatty acids as such or as soaps to freshly prepared cod actomyosin solutions was shown to reduce the solubility, although the variable results obtained with different fatty acids and experimental conditions did not lead to an understanding of the fundamental mechanism of insolubilization. However, Anderson & Steinberg (1964) showed that the natural lipid present in fatty species such as mackerel and dogfish reduced the denaturation of protein in comparison with lean species, and Hanson & Olley (1965) have proposed that protein is protected against FFA insolubilization by the neutral lipid within cells. The amount of neutral lipid in the total lipid of cod flesh was found by Bligh & Scott (1966) to be 14% for whole cod fillets, although only a third of this was triglycerides, with the balance chiefly cholesterol and cholesterol esters. In commercially handled fish free fatty acid was found to be 7% in dark muscle and only 1·8% in white muscle from the same sample. This difference is reasonable in view

of the known greater metabolic activity of the dark muscle (George & Bokdawala, 1964). The lipid-rich Newfoundland trap cod (see above) had no obvious differences in types of lipids (Bligh, private communication) and the rapid initial formation of free fatty acids in comparison with off-shore cod has been ascribed to enhanced enzymatic activity (MacCallum *et al.*, 1964). In lean fish there does not seem to be strong evidence for neutral lipid as the principal factor restricting protein denaturation.

More recently Hamosh, Atia & Shapiro (1966) have demonstrated an esterification process for FFA in fish muscle, which may partially account for the effect of the additional fat in fatty species in protecting against protein denaturation. Previously the solubility of FFA in neutral lipids was thought to be the protective role of the latter in fatty fish. This esterification was inhibited by holding fish muscle at 0°C and muscle which had been frozen and stored at −18°C had a reduced capacity for esterification whereas the total uptake of FFA from the incubation medium was unaltered or even increased. Fish muscle from several species was examined after frozen storage and cod showed the highest uptake of palmitic acid, although this was largely unesterified. In further experiments on the irreversible absorption of fatty acids (as tested by solvent extraction) cod flesh which had been stored at −18°C absorbed significantly more oleic acid than palmitic acid, and carp muscle which had been similarly treated irreversibly absorbed more linoleic acid than palmitic acid, the effect increasing with time in frozen storage. These specific results might be interpreted as showing greater activity towards irreversible absorption on the part of the more unsaturated acids, although this is not true of a variety of fatty acid soaps (Anderson & Steinberg, personal communication, cited by Olley & Duncan, 1965).

Anderson *et al.* (1965) have postulated that protein–FFA binding is the driving force in lipid hydrolysis in frozen fish muscle. This aspect of the complex interactions possible in concentrated solutions of salts, protein, etc., will probably benefit from a new technique, based on nuclear magnetic resonance, for determining the total amount of liquid water present in frozen tissue (Sussman & Chin, 1966). The distinction between freezing of protein-bound and free water should be noted (Love, 1966).

Study of the detailed fatty acid compositions of FFA and phospholipids (Olley & Duncan, 1965), and the concurrent appearance of FFA and disappearance of phospholipids (Bligh, 1961; Olley *et al.*, 1962; Bligh & Scott, 1966) provide firm evidence for the origin of the FFA from phospholipids in teleost species.

In cod muscle stored at −12°C hydrolysis of phosphatidyl ethanolamine was particularly rapid, but ceased after 4 months with one-third of this material still unhydrolysed. Phosphatidyl choline also decreased rapidly for 4 months, hydrolysis thereafter proceeding at a reduced rate (Bligh & Scott, 1966). There is no evidence for the accumulation of lysolecithin in frozen cod undergoing lipid hydrolysis (Lovern & Olley, 1962; Olley *et al.*, 1962; Bligh & Scott, 1966). The FFA produced would be expected to reflect the original fatty acid composition of the phospholipids hydrolysed if oxidation was limited as reported by Dyer & Fraser (1959) for frozen cod, and by

Cardin, Bordeleau & Laframboise (1958) for FFA liberated under the very adverse conditions employed in producing salt cod.

Detailed analyses of the fatty acid composition of lipids and of the FFA formed during frozen storage of the muscle of several species have been reported by Olley & Duncan (1965). This information (cf. Table 1) has been condensed into iodine values (Table 2). Neutral fats in marine fish normally have fatty acid compositions giving iodine values of less than 200 (note the exceptional result for July halibut), while the fatty acids from phospholipids normally have iodine values of 200 or more (Ackman, 1966). Although allowance must be made for the experimental difficulties, the degree of unsaturation of the FFA in the teleost species shows only slightly lower values than in the original phospholipids. Oxidation is unlikely to account for this.

Olley & Duncan (1965) have drawn particular attention to the palmitic/stearic acid ratio (Table 2), which in the teleost species is higher in the FFA than in the parent phospholipids. In the dogfish, where the FFA are derived from neutral lipids (Lovern & Olley, 1962; Olley *et al.*, 1962), this ratio is the same in the neutral lipids and in the FFA. Present knowledge does not indicate whether the unhydrolysed phospholipids are unsuitable for hydrolysis due to fatty acid composition or structure, or whether hydrolysis ceases for other reasons. The possible slightly lower degree of unsaturation in the FFA suggests that differences in fatty acid composition may be responsible, although Olley & Duncan (1965) have suggested that the cessation of hydrolysis may be associated with localized occurrence of particular phospholipids. The difference in the palmitic/stearic acid ratio is considered by these authors to be due to their origin in the phosphatidyl ethanolamine which is probably more rapidly hydrolysed than other phospholipids.

Lipid rancidity and tocopherol

To clarify the lipid hydrolysis–protein denaturation interaction Sheltaway & Olley (1966) recently examined the lipids of North Sea cod at the subcellular level. The recoveries of lipid from fractionation procedures were incomplete except when the experiments were conducted with cod taken in July and August. It was suggested that oxidation during the initial stages of sample manipulation was the cause of lipid loss during the fractionations of cod taken at other times.

Western Atlantic cod have been examined for susceptibility to rancidity by a standardized test involving accelerated oxidation catalysed by Cu^{++} ion added to blends of muscle (MacLean & Castell, 1964; Castell & MacLean, 1964). After storage for 24 hr at 0°C TBA (thiobarbituric acid) values (Castell, Moore & Neal, 1966b) were determined and used as an index of susceptibility to the normal process of rancidity in storage. It is known from these studies (Castell & MacLean, 1964) that Grand Banks and Nova Scotia cod are particularly resistant to this induced oxidation in June and July, and to some extent in August. The more northerly, late-spawning, Strait of Belle Isle cod are still susceptible to induced oxidation in July. This seasonal difference

TABLE 2. Comparison of calculated iodine values and palmitic/stearic acid ratios from the fatty acid compositions* of lipids from several species of fish before and after frozen storage

Sample		Properties of lipid fatty acids					
		Calculated iodine values			Palmitic/stearic ratio		
Species and season	Storage time at −14°C	Neutral	Phospholipids	FFA	Neutral	Phospholipids	FFA
Cod							
March	Fresh	—	255	207	—	4·9	4·6
March	11·5 weeks	—	—	240	—	—	8·1
March	26 weeks	—	266	246	—	1·6	7·3
Lemon sole							
June	Fresh	169	235	—	6·9	3·3	—
December	Fresh	163	—	—	9·0	—	—
December	11·5 weeks	—	—	198	—	—	5·7
February	11·5 weeks	—	—	164	—	—	8·5
Halibut							
July	Fresh	248	265	—	1·05	2·6	—
July	11·5 weeks	—	—	189	—	—	5·5
December	Fresh	182	232	—	3·0	2·5	—
Haddock							
July	11·5 weeks	—	—	223†	—	—	11·4†
July	11·5 weeks	—	—	204†	—	—	10·6†
Dogfish							
December	Fresh	184	210	—	5·9	2·2	—
July	Fresh	—	241	—	—	2·0	—
July	11·5 weeks	—	—	197	—	—	6·0

* From data of Olley & Duncan (1965).

† Values from Beare (1962) are: iodine value of whole lipid fatty acids 201, palmitic/stearic ratio 4·3.

indicates that the resistance to oxidation of North Sea cod lipids in July and August could well have an origin in common with the cod taken in the Western Atlantic in June and July, and the action of the natural anti-oxidant tocopherol has been suggested in this connection (Sheltaway & Olley, 1966; Castell & MacLean, 1964). The few Nova Scotia cod with higher than average lipid content were found (Ackman & Cormier, unpublished results) to contain an average of 3·7 μg of α-tocopherol* per gram of flesh (440 μg/g lipid) as against typical values of 2·3 μg/g (310 μg/g lipid) in winter cod (Ackman & Cormier, 1967). It would be premature to consider these few results as definitely confirming that seasonal tocopherol variations are solely responsible for lipid stability in cod.

In the case of a semi-lean fish such as commercial sole from Nova Scotia waters (largely blackback flounder, *Pseudopleuronectes americanus*) commercial sources indicated that fish landed in June and July were particularly susceptible to the development of rancidity in frozen storage. An experiment was, therefore, set up in which monthly lots of high quality fillets from these sole were handled under normal commercial conditions, half with a fresh water dip and half with a brine dip, through packaging, freezing and commercial storage. Random samples were taken from the frozen packages for determination of lipid, tocopherol and susceptibility to Cu^{++} induced rancidity. Further samples were taken from commercial storage for similar examination after 4 months in frozen storage. The experimental results from the freshly frozen samples showed a change in tocopherol content of the tissue lipid from a maximum of 600 μg/g lipid in April to May to a minimum of 150 μg/g in October to November (Ackman & Cormier, unpublished results). The high relative tocopherol level in April to May corresponded to the annual minimum in lipid content (0·8–1·0%) of the tissue. The highest lipid level (1·4–1·5%) corresponded to a post-spawning period of heavy feeding (July to August), but tocopherol levels were reasonably high in the range 200–400 μg/g lipid at this time.

The notable results from this experiment were that in the period June to July to early August, in sharp contrast to results for the rest of the year, tocopherol disappeared completely from most samples after only 4 months in frozen storage. Equally significant was the observation that although the freshly frozen samples from this period had TBA values characteristic of good quality fish, the samples stored for 4 months gave much higher TBA values than similar samples from other times of the year.

The unexpected result of this experiment with commercial sole was then, that in June and July the commercial sole had a level of tocopherol (*ca.* 300 μg/g lipid) adequate for protection of the fillets against oxidation at other times of the year, but this tocopherol was rapidly destroyed during frozen storage by some other factor, thus permitting oxidation to occur.

The unknown factor responsible for the destruction of the tocopherol could be a combination of circumstances rather than a single entity. For example, the freshly

* α-Tocopheral is the dominant type in marine lipids from higher marine organisms.

frozen samples were actually analysed from 1 to 3 weeks after freezing, and it may be significant that the brine dipped samples tended to have slightly higher tocopherol levels than the fresh water dipped samples in the period May to September, although over the balance of the year there did not appear to be any significant difference. The influence of sodium chloride, either as a pro-oxidant or anti-oxidant, has been reviewed elsewhere (Castell *et al.*, 1966b). In one set of experiments carried out by these authors it was noted that the addition of sodium chloride to blended frozen cod (presumably partly denatured in protein) gave a lesser increase in TBA value after 24 hr than the same addition to fresh cod muscle blends. Moreover, the addition of sodium chloride to fresh cod muscle blends inhibited the development of Cu^{++} induced rancidity at moderate (1–8%) concentrations of sodium chloride.

The dark muscle in salmonids has been shown to be very sensitive to aerobic oxidation when held in a partially frozen condition in sea water enriched with 4% added sodium chloride (Tomlinson *et al.*, 1965). Control samples held at the same temperature in air (25°F) did not show comparable rancidity and it may be inferred that in this case the sodium chloride was a strong prooxidant.

Castell, McLean & Moore (1965) pointed out that sodium chloride may itself require various organic compounds to assist in promoting oxidation. Since the sole are feeding heavily in the period of susceptibility to rancidity other metabolic factors such as amino acids (cf. Castell *et al.*, 1966a) are likely to be involved. An analysis of Nova Scotia cod (late autumn) for muscle tocopherol showed that white muscle (0·7% lipid) had 310 μg tocopherol/g lipid, while the dark muscle (1·85% lipid) had 630 μg/g lipid (Ackman & Cormier, 1967). These results are of interest since the dark muscle in fish is considered more susceptible to the development of normal rancidity due to the presence of haem pigments. The dark muscle has been shown not only to be more sensitive to Cu^{++} in the induced rancidity test, but to give ultimately higher TBA values than white muscle under equivalent conditions (Castell & MacLean, 1964). It should be noted that TBA values for tissue taken from the dark lateral muscle of cod, and also from fresh livers, are subject to large errors. Metals normally present cause oxidation of the acidified material during heating, although this can be controlled by adding ethylenediaminetetraacetic acid (EDTA) or propyl gallate (Castell *et al.*, 1966b). It is known that the levels of many metals, including copper, vary seasonally in fish of various species (Petkevich, 1965), and the potential effect of these metals must be borne in mind in discussing seasonal susceptibility to oxidation (cf. Castell *et al.*, 1965). Unfortunately, adequate data are not available for important North Atlantic commercial species. It is, therefore, impossible to define the significance of the different proportions of tocopherol in relation to lipid in relation to the differing susceptibility to rancidity of the white and dark muscles in cod. Tocopherol is an essential material for animals at the cellular level. It appears that it is necessary to neutralize random peroxide and free radical formation in various cell components and membranes (Dam, 1964; Woodall *et al.*, 1964; Tappel, 1965; Witting, 1965; Roubal & Tappel, 1966). Both light

and dark muscle of cod, as discussed above, are restricted to cellular type lipids. Presumably, the higher tocopherol level in the dark tissue occurs naturally to offset peroxides, etc., formed to a greater extent in this muscle than in white muscle due to the greater metabolic activity of the dark muscle (Braekkan, 1956; Jonas & Bilinski, 1964).

The tocopherol contents of the muscle of a number of other species of fish have also been determined (Ackman & Cormier, 1967). Some of the results, per gram of lipid, were: mackerel dark muscle (4·9% lipid), 310 μg; dogfish dorsal muscle (13·8% lipid) 210 μg; and sablefish dorsal muscle (15·9% lipid), 270 μg. A cod liver (51·2% lipid) had 430 μg tocopherol/g lipid, and the mackerel liver (7·2% lipid) also had 430 μg/g lipid. Many of these figures are similar to those reported by Braekkan, Lambertsen & Myklestad (1963), although these authors also cite much higher values for a number of fish liver oils. Since all marine tocopherol originates in phytoplankton or seaweeds it is not unexpected that an averaging process would take place as the tocopherol is redistributed by zooplankton, small fish and other organisms, finally reaching the commercially valuable large species at a similar level in the depot fats. Presumably, this reserve of tocopherol is available for cellular needs as necessary.

The experiment with the stored frozen sole establishes that the seasonal variations in tocopherol level are not primarily responsible for the development of rancidity in this species. The results of muscle and liver analyses of a limited number of other fish species suggest that tocopherol levels are fairly homogeneous in proportion to lipid for higher species of marine life. The suggestion that tocopherol levels alone figure in the resistance of North Sea cod lipids to oxidation during fractionation, or for the lengthy induction period of Cu^{++} induced rancidity in Western Atlantic cod, both for the same seasonal period (June and July), is however not ruled out by these findings.

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Note on the tempering of fish finger blocks

H. R. SANDERS

Summary. Before blocks of fish fillets are cut by guillotine during the manufacture of fish fingers, their temperature must be raised uniformly. This can be achieved quickly by controlled heat transfer from electrically heated platens.

Introduction

Fish fingers (or fish sticks) are rectangular parallelepipeds of fish flesh sawn or cut from frozen fish fillet blocks, coated with batter, breaded, usually pre-cooked and re-frozen (Dassow, Pottinger & Helston, 1956). The most common raw material is cod.

The first stage in manufacture is the freezing of fillets into blocks of regular shape and dimensions and free from voids. The size and weight of the fish fingers to be produced, and the method of cutting determine the dimensions and weight of the fish block.

In the second stage blocks are sawn or cut into raw fish fingers. In the first cutting operation the block is usually split into three portions by two cuts parallel to the longest sides; the second operation divides each of the portions by cuts parallel to the major faces; in the final operation the strips are cut into individual fingers. Band-saws or gang-saws are used for the first two operations; the final operation may be carried out by a saw or by a guillotine-type cutter.

When saws are used, a strip of about $\frac{1}{16}$ in. is lost as sawdust in each cutting operation. The weight loss in the first two stages is about 5%. If saws are used for the final cut, this results in an additional loss of up to 10% This waste can be completely eliminated by the use of guillotine-type cutters. Unlike saws, these cutters do not work satisfactorily with blocks at normal cold store temperature. Blades wear quickly, the cuts may not be straight, resulting in variable finger weight and the consequent need to increase the mean weight to ensure the correct minimum weights. Blocks also tend to split and crack and the combined losses from these causes may exceed the saving on sawdust.

It is, therefore, necessary to raise the temperature of the blocks from the normal cold-store temperature before the guillotining operation. It is, however, equally important to avoid too high temperatures where losses are caused by fraying and tearing of fingers.

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Blocks at temperatures between 5° and 15°F are generally considered suitable for guillotining.

A special conditioning room maintained at the desired temperature may be used to 'temper' the blocks. At least 48 hr are needed for the temperature throughout the blocks to reach the required value. This long time and the additional space requirement make evident the need for a rapid method of tempering.

A conditioning room with still air at a temperature of 60–70°F may be used with the blocks laid out separately, or the blocks may be passed on their way from the two sawing operations to the guillotining stage through ducts where air at room temperature is blasted over them (Kohr, 1956).

With these methods the danger of overheating part of the surface is, however, always present. It was initially thought that the method of electrical resistance heating (Sanders, 1963) would provide a suitable means for achieving the uniform temperature distribution required for satisfactory cutting. The uniformity of the blocks and the flatness of the major faces lend themselves well to the establishment of good electrical and thermal contact with heated plates. However, preliminary experiments showed that there was no need for the electrical resistance heating stage and conventional surface heat transfer alone was adequate for the purpose.

Materials and methods

Blocks of cod fillets of dimensions $19\frac{7}{8} \times 11\frac{5}{8} \times 1\frac{1}{2}$ in. and weight 13 lb (5·9 kg) were tempered. The heat source consisted of two electrically heated platens, each composed of a heating element 36 × 18 in. sandwiched between 16 SWG stainless steel plates 40 × 20 in. The element extended to within 1 in. of the edge of the platen on three sides, junction boxes were fixed along the fourth side. The nominal rating on the platens was 1 W/in² of element, (0·5 W/in² of heating surface) and in the tests the power supplied to the platens connected in series was controlled at 1·4 kW, equivalent to 1·1 W/in². The voltage across the heaters connected in series varied from 155 to 158 V.

Three blocks were tempered in each series of experiments. Great care had to be taken to limit heat transfer from the surroundings and the experimental blocks were surrounded by other frozen blocks on their journey from the cold store to the place of operation and also during the process. The bottom platen was supported on a layer of frozen blocks. The experimental blocks were placed on the bottom platen touching one another and flush with the three edges of the platen away from the junction box, the top platen was then put on in line with the bottom platen and surmounted by another layer of frozen blocks. To avoid distortion of the top platen, a 48-lb weight was placed at its centre. The heaters were then switched on for the required time. At the end of the heating period the three blocks were quickly stacked with other frozen blocks at top and bottom, and alongside the long edges. It was assumed in the subsequent calculations that the energy was dissipated equally by both surfaces of each platen.

Thermocouples, inserted into previously drilled holes and cut grooves, were used to measure temperatures at the centres of two blocks, at the midpoints of the top and bottom faces of the middle block, and on the top face of the outer block $\frac{1}{4}$ in. from the exposed long edge.

Temperatures were recorded immediately before heat was applied and during the period the blocks remained stacked.

Results

Fig. 1 shows the maximum and minimum temperatures during an experiment on blocks taken from the $-20°$F store and heated for $10\frac{1}{2}$ min. When the blocks had been stacked for 4 min all recorded temperatures were within the required range and after

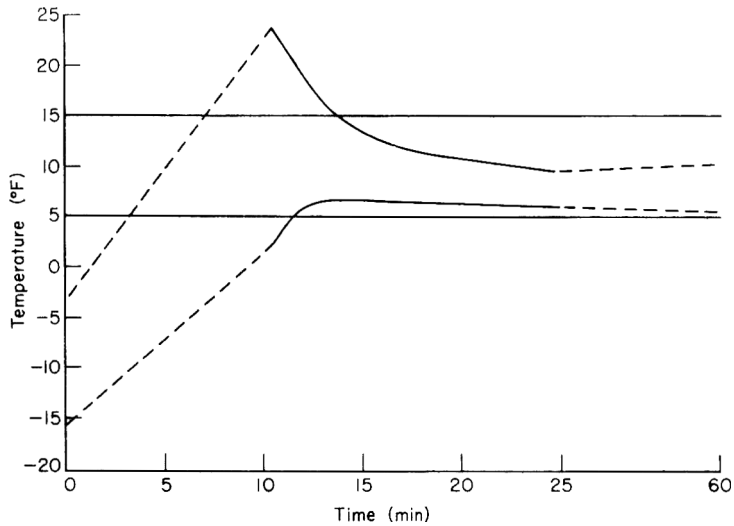

FIG. 1. Temperature range of blocks during processing. Intial temperature $-20°$F.

10 min had settled in the range of $6–10\frac{1}{2}°$F. To determine whether the stacking time is critical the stack was left undisturbed until 1 hr after the beginning of the run. The temperatures were still within the set limits.

The electrical energy supplied to the blocks was 6·9 W-hr/kg. For comparison, the enthalpy of cod at different temperatures is shown in Table 1 re-calculated from Riedel (1956) for zero enthalpy at $-20°$ and $-5°$F.

The electrical energy supplied is only about 70% of the total gain in enthalpy and there was clearly some energy uptake by the blocks from the surroundings in spite of the precautions taken.

The table also shows that an energy input of 10 W-hr/kg will raise the mean temperature of a block initially at $-20°$F to above 5°F, while a block initially at $-5°$F with

TABLE 1. Enthalpy of cod

Temperature (°F)	Enthalpy (W-hr/kg)	
	Zero at −20°F	Zero at −5°F
−20	0	
−15	1·7	
−10	2·5	
− 5	5·3	0
0	7·4	2·1
+ 5	9·7	4·4
+10	12·3	7·0
+15	15·4	10·1
+20	19·5	13·0

the same input will remain below the upper limit of 15°F. In order to test this under practical conditions, blocks were stored at −7°F and then subjected to the same treatment as the blocks from the −20°F store. The results are shown in Fig. 2. It will be

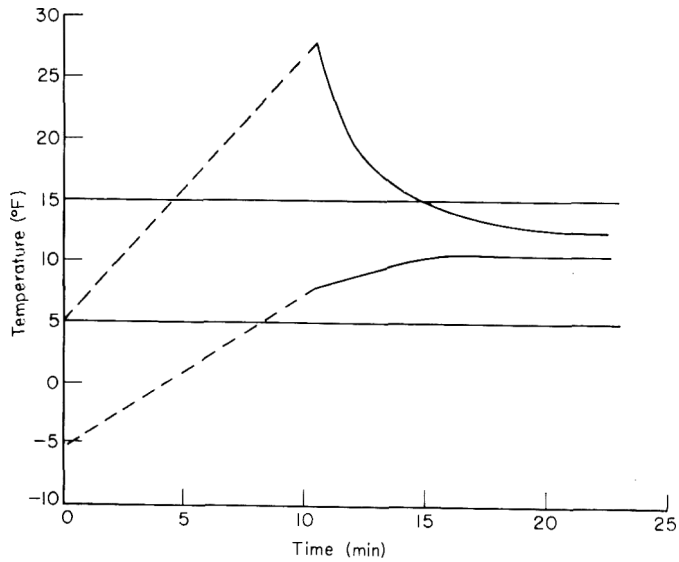

FIG. 2. Temperature range of blocks during processing. Initial temperature −7°F.

seen that the temperatures are again within the limits. In fact the final temperatures are closer to those of the originally colder blocks than expected. This is no doubt due to the greater heat gain from the surroundings in the case of the colder blocks.

Application

In the following calculations a throughput of 2 ton/hr (360 blocks/hr) is assumed.

For a 10-min heating cycle sixty blocks must be heated at any one time. If each layer consists of four blocks, the resulting pile of fifteen layers has overall dimensions 40 in. long by 20 in. deep by 22½ in. high, ignoring the thickness of the platens. If two such assemblies are used, one can be loaded and unloaded manually or automatically while the other is in operation. An arrangement similar to the existing horizontal plate freezers would be suitable. As the platens can be designed to give the required power at mains voltage or a submultiple thereof, no transformer is required and the only special electrical equipment needed is a switch, preferably an adjustable time-switch.

The power required is 14 kW and the cost of electricity taking into account maximum demand charge and unit charge is between 1s 8d and 2s 4d per hour depending on the tariff, or about 1s per ton on average.

Conclusion

By the use of electrically heated platens, 1½ in. thick blocks of fish can be brought from −20°F to a temperature suitable for cutting into fish fingers in 20 min. A variation in initial temperature of 15°F can be accommodated. The process is easily controlled and costs are low.

Acknowledgments

The work described in this paper was carried out as part of the programme of the Torry Research Station.

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J. Fd Technol. (1967) 2, 9P–14P.

PROCEEDINGS OF THE INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY

Symposium on Meat and Meat Products held jointly by the Northern England Branch and the Institute of Meat at the Blackpool College of Technology, Wednesday, 5 October 1966

Technology has been described* as 'the interdigitation of science with industry' and this symposium was an admirable example of technology so described.

The meat industry is an exceptionally difficult one to interdigitate with, because it is, of all food industries, the one most traditionally based and least amenable to penetrating enquiry.

The structure of the symposium was simple—a single speaker from the trade itself, one from science in industry, and one from research. Much depended therefore on the choice of the three spokesmen. The Branch and the Institute of Meat are to be congratulated on the choice they made.

The three papers have already been published in the *Bulletin of the Institute of Meat:* Mr Geary's in the October/November issue (1966, No. 54, pp. 3–10); Dr Kitchell's in the January issue (1967, No. 55, pp. 6–13); and Mr Williams' in the May issue (1967, No. 56, pp. 17–25). The present account is therefore a brief summary of their papers and of the discussion to which they gave rise.

The first paper, **The Future of Meat Trading** was given by *Mr David J. Geary*, Managing Director, Messrs Payne & Son (Butchers) Ltd. He began with a searching analysis of the troubles besetting the retail butcher, forthrightly indicting the less-than-honest advertizing of standardized products on the one hand, and the uncultivated taste of the acquiescent customer on the other. He saw the answer to lie in effort on the butcher's part to recover the attractiveness of good meat, skilfully prepared and presented, and to restore awareness in his customers of genuine quality in the meat he offers them.

This sentiment was later emphasized in the discussion by *Mr P. A. Henderson*, who felt (as others in the audience may well have done) that Mr Geary's strictures on the manufacturer and the housewife had been rather severe. He, also, felt that responsibility for educating the housewife lay with the butcher. In reply, Mr Geary said that although this was hardly the butcher's responsibility, it was obviously very much to his interest to do so.

Mr Geary went on to discuss the more fundamental reasons for the change of fashion in meat consumption during the last decade. He equated this with an actual decrease

* *J. Fd Technol.* (1967) 2, 13

in the physical need for fat in the form of meat, the reason for this being two-fold: an increased supply of fats in other and cheaper forms—butter, margarine, chocolate and synthetic creams; and a lower calorie requirement due to lighter physical work and more efficient space heating. (It is, in fact, not generally appreciated that carcase meat contains much more fat than protein; that it is a high-fat even more than it is a high-protein component of the diet.) The problem, therefore, as he saw it, is to provide the flavour and succulence in lean meat without the finish that the best quality meat traditionally required. He could not see this being done with the present-day practice of barley beef and broiler chicken. His final shaft was directed at modern line-slaughtering techniques: 'We have adopted the American method. Unfortunately we have not adopted the American efficiency that goes with this method.'

In the next paper, **Trends in Meat Technology,** *Mr E. F. Williams*, Chief Chemist Messrs J. Sainsbury Ltd, took up this last implicit challenge. The slaughtering procedure he has introduced might, with justification, be described as a British method, since it is based on the research of mainly British workers, from 'the early 1900s when the pioneering studies of Callow showed that a number of quality defects in pork could arise as a result of pre-slaughter stress and post-slaughter handling'.

Mr Williams emphasized the value of high-pressure water sprays in reducing the bacterial load on carcases, and immediate chilling of carcases in reducing loss of weight by evaporation. These prescriptions gave rise to several questions. *Dr Harwood* asked whether there was any evidence that spraying of pigs with cold water prior to slaughter reduces the incidence of watery muscle; and whether there was any danger that refrigeration at high humidities leads to incidence of mould growth on carcases. Mr Williams replied to the first question that the work of Briskey and his co-workers and their own work on pork clearly shows that this results in a reduction in watery pork. It is now normal practice in Russia and Yugoslavia to spray pigs with cold water for at least 1 hr prior to death; and to the second, that under correct refrigeration conditions there is no evidence that high humidity leads to mould growth in carcases.

In reply to a question from *Dr Bate-Smith*, he said that the cost of removing heat by refrigeration was much less than that represented by the loss in carcase weight if heat were allowed to be lost by evaporation.

In reply to *Mr Toseland*, he said that for retail pork three-quarters of the carcase weight so saved was retained up to the point of sale.

Mr Williams answered questions from *Mr D. Geary* and *Mr D. I. Brown* by emphasizing again the effect of wiping cloths in spreading contamination on the surface rather than removing it, and the need for following water spraying with adequate refrigeration if the advantages of this treatment are to be exploited.

He made a special point in his paper of the importance of avoiding stress during transport to the slaughterhouse, and this gave rise to several questions. *Dr Kapel* asked whether there was any danger of tranquilizing agents, if these were introduced, being carried over in the meat to end-users; the reply being that clearly we could not use

any form of tranquilizer in which there is a carry-over of degradation products. Substances such as succinylcholine could be used as they are rapidly metabolized. *Mr C. B. Casson* asked whether it was likely that the injection of animals with enzymes prior to slaughter would become established in practice. Mr Williams thought that with the enzymes at present available the effect was too uneven over the carcase, some parts being tenderized too much giving rise to meat of poor texture.

Preparing the way for Dr Kitchell, Mr Williams ended up by emphasizing the need for more research into the means of achieving 'a very substantial improvement in the bacterial quality of meat and a greater knowledge of those colour changes which do not stem from contamination'.

The subject of the paper by *Dr A. G. Kitchell* (Meat Research Institute, Low Temperature Research Station, Cambridge) was **The Microbiology of Pre-Packaged Meats.** He dealt immediately with Mr Williams' last point. 'The preferred conditions of packaging are not the same for different kinds of meat, nor for different aspects of quality, thus requirements may be mutually exclusive. For fresh beef and pork, access of oxygen is thought necessary for a bright red colour. For poultry this consideration is less important and it is permissible to pack in impermeable wrappers even though (as with other meats) the tissue respiration soon consumes much of the contained oxygen. With cured meats, where the pigment has been fixed by combination with nitrite, oxygen is precluded to prevent the colour fading. As oxygen has a very important influence on micro-organisms, favouring the growth of some (aerobes) and hindering others (anaerobes), different situations arise in the different cases: the bacteria on fresh meats, wrapped in oxygen-permeable wraps, are not, it might be supposed, subjected to very abnormal conditions; those on an anaerobically wrapped chicken are. With sausage, the most important requirement is that the wrapper should be freely permeable to water vapour, for condensation on the sausage encourages loss of bloom and the formation of slime.'

Dr Kitchell dealt with each of the classes of meat in turn. That mere numbers are less important than kinds of bacteria was strikingly brought out in his consideration of spoilage in cured meats. Here again (as in Mr Williams' paper) the importance of pH is evident, but salt content, nitrite and smoke all play a part in controlling the growth—and masking the effects—of the microflora. Conditions favouring the growth of lactobacilli, as compared with micrococci, are preferred, and the present trend of research is to explore what these conditions are.

In one of several questions to Dr Kitchell, *Mr T. McLachlan* was especially concerned with the effects of heavy smoking on the conditions prevailing in packaged bacon: the concentration of salt due to the removal of 5–5½% moisture; high formaldehyde and phenol absorption. This gave Dr Kitchell the opportunity to affirm his belief that the effectiveness of smoke is due more to its volatile acid constituents than to formaldehyde or phenols; and that the differences between green and smoked are much more important than those between lightly and heavily smoked bacon.

Mr Geary asked whether the effects of micro-organisms on flavour had been looked at, and also what was the effect of changing the natural distribution of organisms. Dr Kitchell replied that many attempts had been made to correlate the observations of taste panels with bacteriological counts, but, with the exception of spoilage off-flavours, with little success. His own results with pre-packed lamb clearly showed what the effects could be of domination of the microbial flora by one particular organism, but the influence of packaging is, at the moment, rather unpredictable.

Mr D. E. Smart asked about the growth of *Clostridia* in vac-packed cooked meat. Dr Kitchell said he had little to add to what he had said in his paper, namely that in experiments with uncooked meats stored up to 15°C they had never yet observed development of *Clostridia*. He thought that with cured meats the influence of curing ingredients and smoke was likely to be more important than that of packaging.

E. C. BATE-SMITH

Report of paper: 'What a Chairman Expects of a Food Technologist'

W. ROWAN HARE

Chairman, J. & J. Colman Ltd; also Chairman, Food Manufacturers Federation

(*Presented at the Annual General Meeting of the Institute*, 6 *December* 1966)

Mr Hare began his lecture by considering a young man with a degree or an equivalent qualification in a relevant scientific subject, and with adequate potential of being a good leader, entering the Food Industry as a Food Technologist. He made it clear that from the very start of his career a great deal will be expected from this young technologist; he must be articulate, both in speech and on paper, and must be extremely versatile as food scientists are involved at all the links of the food chain, leading from the primary producer to the consumer. All the scientific disciplines, and many of the humanities, are involved as well as the engineering sciences although it does often seem that there is sufficient common background to the basic scientific subjects, for a man trained in one of them to have a good chance of picking up any of the others.

It is essential that a food technologist should understand the profit motive as most young technologists today have no idea of how industry is run and often they do not realize that a profit must be made. It is also necessary that a technologist is extremely energetic and mentally as well as physically agile. It has been said that the energy and performance of people differs at least as much as their I.Q.s, and also that you cannot improve a person's energy, you can only make the best use of what he has got.

It must be realized that university education is very expensive and that the individual should, therefore, strive to make full use of it. In the latter part of university life students can obtain much valuable information from the University Appointments Board, who can advise them about their prospects in any particular field and can attempt to put them on the right track. It is important that an employer does all he can to receive this new recruit smoothly into the industry, by assisting over the initial hump and by making

certain that he appreciates the functions of the various component parts of the industry in relation to his own job and to the possibility of future promotion. It is necessary to ensure that he has a clear understanding of the definitions of positions used within his company, because these definitions can vary considerably throughout the industry. During the initial period the employer must show a continued interest, and must ensure that he has proper training and is given further encouragement by allowing release for use of the relevant scientific societies, by helping him to make contacts and by giving him the chance to travel. He can also assist by organizing discussion both on topics relevant to the work being carried out and on fringe subjects, in order to ease the increasingly difficult task of keeping up to date with published literature.

Mr Hare then said a few words concerning certain of his own companies' problems and briefly outlined their approach to Research and Development. Their utilization of Critical Path Analysis methods greatly improved the translation of development department specifications into viable production units, but he emphasized the need to introduce this system right through the chain of command and recommended that initially it should be introduced through outside experts.

Glancing into the future Mr Hare said that if Britain enters the Common Market the food industry must be prepared for a radical revolution and our versatile technologists must have their eyes directed to the food problems of the world in 2000 A.D., and must be prepared to study a couple of languages as well as scientific subjects.

Discussion

Mr S. Cakebread asked the Speaker where he thought the time was coming from to read foreign literature, having studied and learned the language, as well as the vast amount already printed in English. The Speaker replied that he fully appreciated this difficult problem of reading all relevant literature and he suggested that it should be controlled by the Head of the Research and Development Department, perhaps by allocating time each week to reading and fringe discussions.

Mr McFarland said that in addition to all the requirements of a food technologist, suggested by the Speaker, he thought that a certain amount of 'salesmanship' was necessary. In reply, the Speaker said he agreed with this point whole heartedly and added that although salesmen often get adequate product training, technologists rarely get any grounding in any marketing aspects.

Mr J. Blanchfield continued on this point by agreeing that a technologist should know something of the sales side of the business and by adding that he felt a technologist should, himself, be a salesman, capable of 'selling' his results and findings for their optimum utilization, both in policy making and in manufacturing operations.

Mr R. Butler followed up a point on communications and said he regretted that so many Directors only pay lip-service to the ideas of communication. He then suggested that the Programmes Committee should arrange a meeting to discuss 'Marketing' and

'Sales' as there seemed to be a great deal of confusion regarding the meaning of these terms.

Mr J. Demmar asked if the versatile food technologist, with his knowledge of the production and development aspects, could apply himself to the more senior general management positions. In reply the Speaker said that this was certainly possible and he hoped that the scope of the food technologist would reach even further than general management positions.

Mr Rice, a student studying food science, said, that in his course, approximately 4–5 hr each week was diverted towards liberal study lectures which he thought could be better spent studying some technical aspects in more detail. In reply the Speaker said that he had tried to stress the need for 'versatility' by indicating the broad scientific background required to cover the wide variety of aspects likely to crop up, but it was also necessary to be able to appreciate many of the non-scientific aspects like management problems and accountancy.

P. B. KINGDON

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Book Review

Industrial Food Preservation (Industriel Levendsmiddel-Konservering) Vol. 1, **General,** Pp. xv + 477; Vol. 2, **Cold Preservation,** Pp. x + 360; Vol. 3, **Heat, Radiation, and Chemical Preservation,** Pp. xii + 518. By E. ANDERSEN, M. JUL and H. RIEMANN, with editorial assistance by J. E. PEDERSEN.

Copenhagen: Teknisk Forlag, 1965. Dkr. 294 (approx. £15).

For the English-speaking community, it seems a pity that the largest and most comprehensive work yet to appear on industrial food preservation has been written in Danish and it is to be hoped that the authors, who all speak and write fluent English, will be persuaded to bring out an English edition before very long. On the other hand the book was intended for use in Danish universities and technical schools by students, some of whom do not speak fluent English and who already probably find it difficult to pursue their ordinary course.

As so often happens, with works of this kind, many years have been spent in its preparation and some revision would be required for a further edition. While the book is extremely comprehensive, moreover, it has been written for Danish students with a bias towards Danish foods, so that a revision for the American and English public would entail a considerable amount of further work, although not quite as extensive as might be thought at first because the Danes have for many years concentrated to a considerable extent on the British and American markets, and the whole subject of food has been dealt with from the world standpoint. The first chapter deals with the world supply and demand for food and the authors are rather pessimistic about the future unless more rapid steps are taken to improve agriculture and more especially the fishing industry. Since about 45% of all foods are perishable and 20% of all foods are lost through improper preservation or storage it is essential to reduce this loss to an absolute minimum. The effects of micro-organisms and pests on foods are discussed at length and also the best ways of dealing with them from the point of general hygiene.

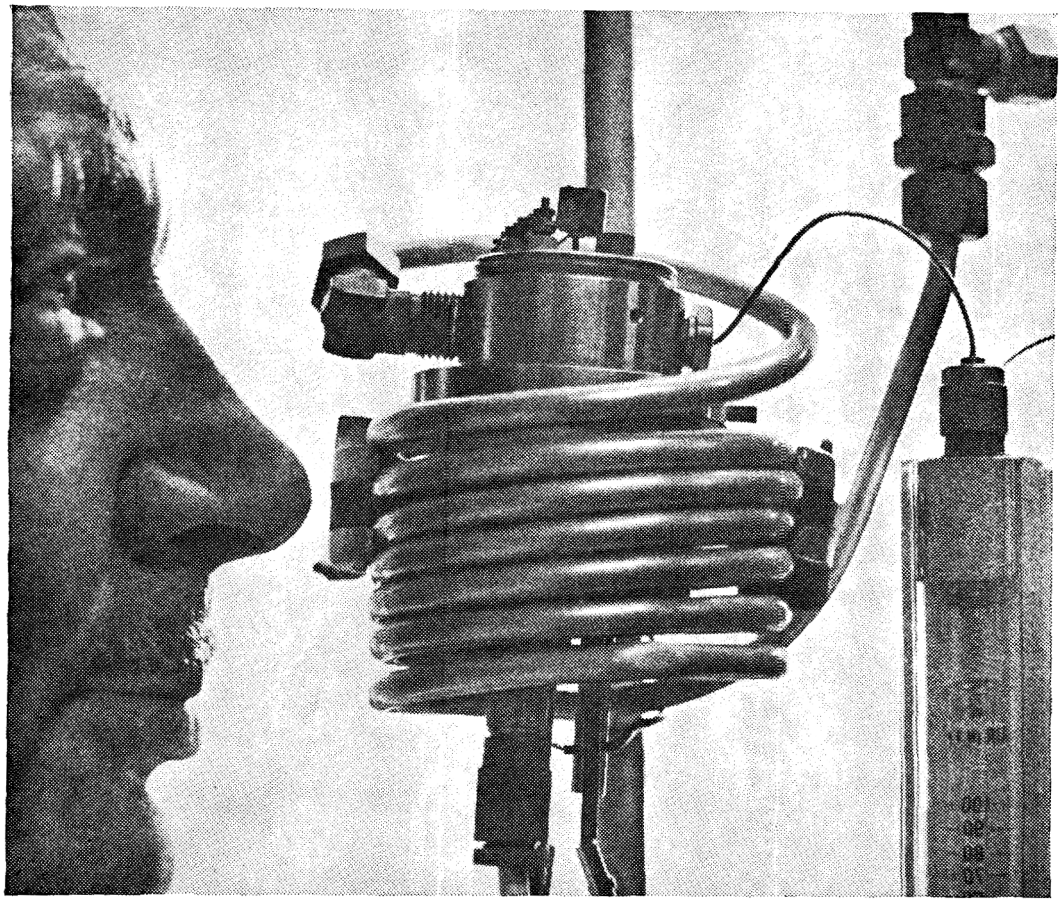
With the growth of industrialization about 80% of all foods are processed and have been for many years, but the methods of processing are changing and the housewife is now demanding a much larger supply of pre-packed and convenience foods. The effect of preservation on the nutritive value of foods is discussed and it is shown that with proper care there is little loss, but that by processing in bulk there is a general saving of food material that would otherwise be wasted, and that, for instance in America, if foods were generally treated and cooked by traditional methods in the home their cost would increase by about 7%.

The authors believe that food preservation begins with its initial production and,

therefore, discuss the influence of feeding and breeding, pre- and post-slaughter changes, and the need for care in seed selection and the harvesting of the final fruit or vegetable. Considerable space is devoted to methods of fishing and the changes that take place immediately after capture, but the authors also discuss the transport and keeping of live fish including the preparation of artificial seawater and its filtration and aeration.

As would be expected of a team which originally met to work together at the Meat Research Laboratory at Roskilde, the sections on meat products are very good and some space is also devoted to marinades and other semi-preserves. A considerable amount of detail is included about the construction of plant, basic principles of formulae for food products, and even the toxic concentrations of gases employed for refrigeration.

T. McLachlan



Nose...meets...automatic nose

As any dog will tell you, the human nose is a sensitive but imprecise instrument. In other words your nose can pick up a 'scent' but not discriminate between scents, as a bloodhound can.

But even a prize bloodhound would not be able to equal the degree of discrimination of the apparatus *in the picture*. Called a gas chromatograph, it is used by Metal Box Packaging Research to determine the characteristics of aromas. Gas chromatography is just one of many research tools used by Metal Box to control the quality of packaging.

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Light engineering and marketing organisation with 38 factories in Britain, 32 overseas.

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A youthful and vigorous industry with tremendous potential for the future.

MB *There's more to Metal Box than metal boxes*

– they're leaders in research into metal, paper and plastic packaging

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INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY

TENTATIVE PROGRAMME, 1967

Date	*Time and Place*	*Subject*	*Speakers*
13 July	J. Lyons & Co. Ltd, 149 Hammersmith Road, London, W.14 2.30 p.m.	Factory visit Bakery and Central Laboratories	
28 September	Safeway Food Stores Ltd, 159–165 Edgware Road, London, W.2 6.30 for 7.00 p.m.	Ladies' evening 'Open house'	
3 and 4 October	Belfast (North Ireland Branch) 2 days	Symposium: Food Processing	
17 October	Weybridge National College of Food Technology (Joint with Students' Society) 7.00 p.m.	Heat Processing	Mr A. Hersom, Research Manager, Cross & Blackwell Ltd.
October	Salford (Northern Branch) All day	Symposium: Baking and Bakery Products	
1 November	National College of Heating, Borough, S.E.1 6.30 p.m.	The Function of a Development Department	
8 November	Food Group (Joint with I.F.S.T.) 5.45 for 6.15 p.m.	Codex Alimentarius	Mr J. H. V. Davies
27 November	Glasgow (Scottish Branch)		
5 December	Royal Society, London 6.15 for 6.45 p.m.	Annual General Meeting	Mr J. H. Hulse, F.A.O., of U.N.

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Typescripts (two complete copies) should be sent to the Editor, Dr E. C. Bate-Smith, A.R.C., Institute of Animal Physiology, Babraham, Cambs. Papers should be typewritten on one side of the paper only, with a 1½ inch margin, and the lines should be double-spaced. In addition to the title of the paper there should be a 'running title' (for page headings) of not more than 45 letters (including spaces). The paper should bear the name of the author(s) and of the laboratory or research institute where the work has been carried out. The full postal address of the principal author should be given as a footnote. (The proofs will be sent to this author and address unless otherwise indicated.) The Editor reserves the right to make literary corrections.

Arrangement. Papers should normally be divided into: (a) Summary, brief, self-contained and embodying the main conclusions; (b) Introduction; (c) Materials and methods; (d) Results, as concise as possible (both tables and figures illustrating the same data will rarely be permitted); (e) Discussion and conclusions; (f) Acknowledgments; (g) References.

References. Only papers closely related to the author's work should be included; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors all names should be given when cited for the first time, and thereafter only the first name, adding *et al.*, e.g. Smith *et al.* (1958). The '*et al.*' form should always be used for works by four or more authors. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Smith *et al.* (1958a). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention (a) name(s) of the author(s); (b) year of publication in parentheses; (c) title of journal, underlined; (d) volume number; number of first page of article. References to books and monographs should include (a) name(s) and initials of author(s) or editor(s); year of publication in parentheses; (b) title, underlined; (c) edition; (d) page referred to; (e) publisher; (f) place.

Standard usage. The *Concise Oxford English Dictionary* is used as a reference for all spelling and hyphenation. Verbs which contain the suffix ize (ise) and their derivatives should be spelt with the z. Statistics and measurements should always be given in figures, i.e. 10 min, 20 hr, 5 ml, except where the number begins the sentence. When the number does *not* refer to a unit of measurement, it is spelt out except where the number is greater than one hundred.

Abbreviations. Abbreviations for some of the commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular unless confusion is likely to arise.

gram(s)	g	second(s)	sec
kilogram(s)	kg	cubic millimetre(s)	mm^3
milligram(s)		millimetre(s)	mm
(10^{-3} g)	mg	centimetre(s)	cm
microgram(s)		litre(s)	l
(10^{-6} g)	µg	millilitre(s)	ml
nanogram(s)		pound(s)	lb
(10^{-9} g)	ng	gallon(s)	gal
hour(s)	hr	milliequivalent	mEq
minute(s)	min	R_F values	R_F

Figures. In the text these should be given Arabic numbers, e.g. Fig. 3. They should be marked on the backs with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of a figure the top should be marked with an arrow. Each figure must bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Line diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper and should be about four times the area of the final reproduction. Lines and lettering should be of sufficient thickness and size to stand reduction to one-half or one-third. Letters and numbers must be written lightly in pencil. Whenever possible, the originals of line diagrams, prepared as described above, should be submitted and not photographs. The legends of all the figures should be typed together on a single sheet of paper headed 'Legends to Figures'.

Tables. There should be as few tables as possible and these should include only essential data; the data should not be crowded together. The main heading should be in capitals with an Arabic number, e.g. TABLE 2. Each table must have a caption in small letters. Vertical lines should not be used.

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Contents

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