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The Structure and Composition of Skeletal Muscle

By G. Hamoir* and H. A. McKenzie†

STRIATED skeletal muscle makes up 30–40 per cent. of the body weight in higher vertebrates. It represents a greater proportion of the weight than any other tissue. In a man of 70 kg the weight of the muscle is about 30 kg while that of the next tissue, the bones, amounts only to 10 kg. These muscles may be red or white, the red colour being due to myoglobin. A mammalian muscle is enclosed in a sheath. Inside this sheath are the very elongated muscle cells called fibres, and between these fibres are fat deposits and connective tissue. The muscle fibres which form the contractile tissue have lengths of several centimetres and diameters ranging from 0·01 to 0·1 mm. They fuse at the ends with the tendon fibrils (Fig. 1). Each fibre possesses an enclosing membrane or sarcolemma (see Barer 1948; Bailey 1954, 1955; Perry 1956). In some muscles such as the psoas, the fibres are parallel to the long axis of the muscle, in others, such as the gastrocnemius, they are inclined.

The transverse striations are due to alternating bands of materials of different refractive indices, the anisotropic A-band appearing bright under polarized light, and the isotropic band dark. Under ordinary light the reverse situation holds. The contractile material of the muscle is located inside the muscle fibres in the form of thin longitudinal fibrils, called myofibrils, approximately 1μ in diameter, packed closely together across the width of the fibre and extending along its whole length. The fibrils are separated from each other by a sarcoplasmic gap about 0·5μ across. Under the electron microscope the fibril appears to be composed of thin threads called filaments. The arrangement of the latter is shown in Figure 2. The thick filaments correspond, according to Hanson and Huxley (1955), to myosin; the thin ones to actin. In the H-zone, the thin filaments are not visible in transverse sections. Filaments should, however, exist joining the thin actin filaments as shown in Figure 2. When an isolated myofibril is treated with a solution able to extract myosin selectively, the anisotropic bands become less dense and their birefringence disappears, but the myofibril does not fall to pieces at the H-zone. Something other than myosin must exist in this region to keep the myofibril together.

The muscle cell contains also two other main types of formed elements, nuclei and granular bodies. Nuclear material accounts

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* Dr. Hamoir, who is the Director of the Laboratoire de Biologie générale, Université de Liège, Belgium, visited Australia in 1958 to report on research in muscle biochemistry in the Division of Food Preservation and Transport, and to carry out investigations on myosin in fish muscle in collaboration with Dr. H. A McKenzie, at that time leader of the Division's Physical Chemistry Unit.

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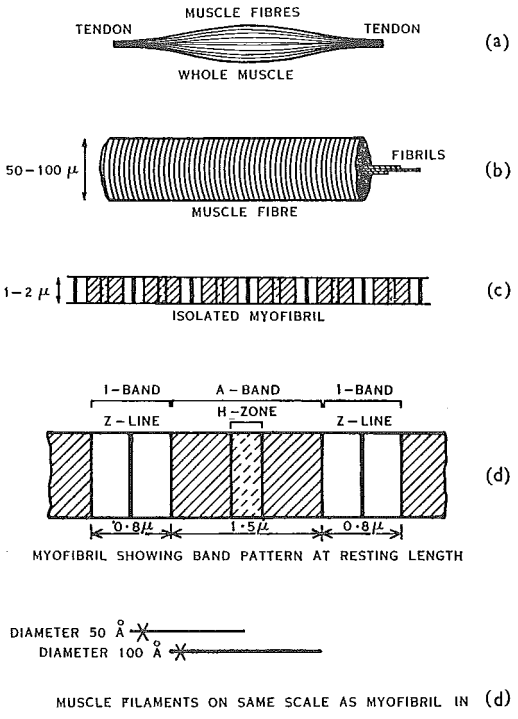

Fig. 1.—The structure of muscle at different levels of organization. The dimensions shown are those for rabbit psoas muscle. (After H. E. Huxley 1956b.)

for only a very small fraction of the total dry matter of adult skeletal muscle. The proportion of granules is much larger but varies greatly from one muscle to the other: it represents 3–4 per cent. of the total protein nitrogen in rabbit muscle and 15–20 per cent. in pigeon breast muscle (see Perry 1956). A schematic representation of the components of the muscle cell is shown in Figure 3.

The occurrence between the muscle fibres of vascular, nervous, and connective (elastin and collagen) tissues as well as material of the interstitial space should also not be neglected in the study of whole muscle. More detailed information on muscle structure at different levels or organization can be found in the reviews of Barer (1948), Hanson and Huxley (1955), Hodge (1955), Sjöstrand (1955), Hamoir (1956), A. F. Huxley (1956), H. E. Huxley (1956*a*, 1956*b*, 1957) and Szent-Györgyi (1958).

CHEMICAL COMPOSITION

In considering the chemical composition of muscle it is well to remember that the composition, especially the lipid and glycogen content, is influenced by the physiological state, and that the muscle cells do not represent the total volume of muscle. There is an important extracellular space which has received little attention (see Harris 1956). In frog, cat, and rat muscles, it amounts to 12–15 per cent. of the total volume; it can be divided into:

- The interstitial space which occupies 9–12 per cent. of the total volume, and
- The capillaries which correspond to about 2–3 per cent. of the total volume (Boyle *et al.* 1941).

Human plasma has a protein concentration of 7–8 per cent. and the concentration in lymph is about 2–3 per cent. Pronounced differences in ionic concentration also occur: K^+ is intracellular, Na^+ is mainly localized in the blood and the lymph, Cl^- is entirely or almost entirely in the blood and the lymph. It is important in the comparison of different muscles to take account of possible differences in extracellular space.

The general composition of skeletal muscle refers also to the total: capillaries+interstitial space+muscle cells. Thus it gives only an approximate idea of the composition of the muscle cells. Corrections should be made for proteins such as serum albumin or haemoglobin and, in general, for components having different concentrations in blood and in the cells.

The general composition of rabbit skeletal muscle is given in Table 1, but the accuracy of the determinations is very variable. The partition of the protein nitrogen in rabbit muscle is well known, and we know fairly well the water and glycogen contents. However, other data are still missing and the values given in Table 1 for lipids, nitrogen extractives, salts, and protein are very approximate ones based on general knowledge of the chemical composition of skeletal muscle. In fact, a complete analysis of a well-defined muscle has never been worked out.

According to these values, the lipid and glycogen contents would amount only to 3–5 per cent. of the fresh weight or 15–25 per

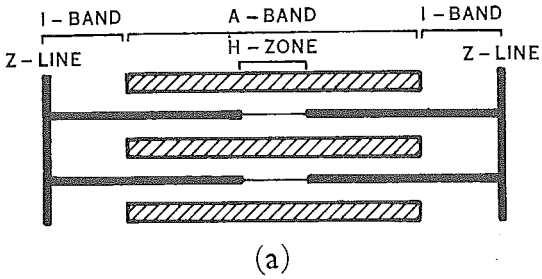

Fig. 2.—Arrangement of filaments in striated muscle: (a) *longitudinal,* (b) *sectional.* (*After H. E. Huxley 1956*b.)

cent. of the dry weight. The proteins amount to about 70 per cent. of the dry weight of muscle. They are divided into three groups according to their behaviour in the presence of various extractants (see Table 2). The sarcoplasmic proteins are soluble at low ionic strength and in them we can distinguish an albumin fraction (myogen) and a globulin fraction (globulin X). The structural proteins which form the myofibrils are extracted at an ionic strength equal to or greater than 0·35, and neutral pH; they include myosin (which is the major component of the muscle cell), actin, and tropomyosin. The proteins insoluble in dilute hydrochloric acid or sodium hydroxide form the third fraction, the stroma. The content of rabbit skeletal muscle in these different groups of proteins is fairly well known. The values given are those determined by Hasselbach and Schneider (1955).

Variations can occur in the protein composition of muscles of different origin. One factor to consider is the colour of the muscle. It is well known that red muscles differ from white ones. The volume of the capillaries is apparently higher in red muscles. As noted earlier, their content of cytoplasmic granules is also higher. The data of Hasselbach and Schneider obtained with a mixture of white and red muscles represent mean values; it is certain that a higher stroma protein content would be found in rabbit red muscle. On the other hand, fish muscle has a very low stroma protein content; it amounts to only about 3 per cent. in bony fishes and to 10 per cent. in the cartilaginous ones. In fish the actomyosin content appears higher than in rabbit muscle (Hamoir 1955*b*). The protein composition of myogen from white and red carp muscle also differs widely (Hamoir 1955*a*). Some of these variations induce important metabolic changes. Lawrie (1953*a*, 1953*b*) has shown that the oxidative activity of muscle is directly related to the granular content. In ordinary biochemical work, however, these variations have been neglected.

LOCALIZATION OF CONSTITUENTS

The localization of some of these constituents in the structures of the muscle has already been described, but it is interesting to consider more closely the relation existing between the analytical data and the morphology of the muscle. The sarcoplasm contains the soluble components of the cell: the salts, the nitrogenous extractives, and the proteins soluble at low ionic strength. The latter constitute about 30 per cent. of the total proteins i.e., 4–6 per cent. of the fresh weight. Haumann and Weber (1935) have shown by a dilution method that this fraction can be dissolved in only one-fifth of the fibre volume. There is now independent evidence for this. The observation of living frog muscle with the interference microscope has shown that the protein concentration in the sarcoplasm is higher than in the myofibril (Huxley and

Table 1—Chemical Composition of Rabbit Skeletal Muscle

The values apply to rabbit skeletal muscle having the general composition: capillaries 2–3%+extra-cellular space 9–12%+muscle cells 86–88%

Component	Percentage of Total Fresh Weight
Water	75–80
Lipids	2
Glycogen	1
Salts	1
Nitrogenous extractives	1
Proteins	15–20

Niedergerke 1954), and amounts to about 25–30 per cent. As myogen solutions of 40 per cent. concentration are not unduly viscous, the sarcoplasm is still fairly fluid. Myogen is a very complicated mixture of proteins, mainly the enzymes of glycolysis as well as others involved in general aspects of cell metabolism. Their number has been estimated as over 50.

The muscle sarcoplasm contains also particulate components. Glycogen occurs in granules, and according to early histological work, fat droplets appear to exist in sarcoplasm. Granules are also present with diameters ranging from 0·03 to 1·0μ. They contain the enzymes of the succinic dehydrogenase-cytochrome system—a latent ATP-ase activity, and according to Portzehl (1957) the Marsh factor, which inhibits the hydrolysis of adenosine triphosphate (ATP) in resting muscle. Thus these muscle components play an important role in the activity of the muscle cell which does not appear limited to the oxidation of metabolites with concomitant production of ATP. Like mitochondria, they consist of a phospholipid-protein-nucleic acid complex.

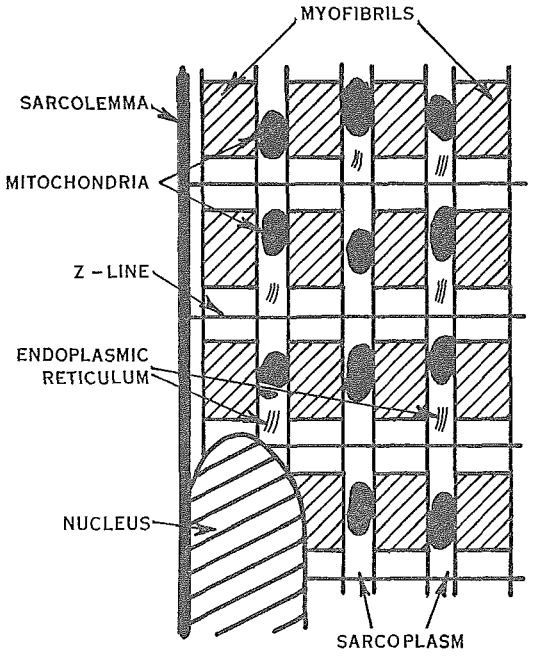

Fig. 3.—Components of skeletal muscle cell. (After Perry 1956.)

The myofibrils appear as a semicrystalline protein gel which occupies about 65 per cent. of the volume of the fibre. Their average protein concentration is 15–20 per cent. The localization of myosin in the myofibril is now well established. The relative proportion of the thin and the thick filaments is also in agreement with the amounts of actin and myosin present in muscle as shown by interference microscopy (Huxley and Hanson 1957) and biochemical determinations (Hanson and Huxley 1957). The localization of tropomyosin in the myofibril is still unknown.

INTERPLAY OF MUSCLE CONSTITUENTS

It is currently believed that actin and myosin in an unstimulated living muscle are prevented from combining by the presence of ATP, which in some way occupies or masks the actin-binding sites on the myosin molecules. The long-range elasticity exhibited by such a fibre is considered to be due to the absence of actin-myosin linkages. Under these conditions ATP has a "plasticizing" effect. When the fibre is stimulated, ATP is dephosphorylated to ADP under the influence of the myosin ATP-ase; actomyosin is formed and the fibre exhibits normal, not long-range, elasticity. It may shorten and exert tension. The energy for contraction is considered to come from the splitting of the ATP. Marsh (1952) has shown that in the unstimulated fibre the ATP-ase activity of myosin is inhibited by a "relaxing" factor. Stimulation of the contractile system is considered to result in inactivation of the relaxing factor. The nature and mode of action of the factor have been examined recently by Bendall (1958), Molnar and Lorand (1959), Weber (1959) and Gergely (1959). This interplay of the different muscle constituents involved in the contraction has been well summarized by H. E. Huxley (1956*a*, 1956*b*) in the following sentences:

The contractile structure is built largely out of two proteins, actin and myosin.

Some sort of combination can occur between actin and myosin.

This combination is modified by the presence of ATP.

Myosin is an enzyme for the dephosphorylation of ATP.

Table 2—Proteins from Rabbit Skeletal Muscle

	Description of Protein		Percentage of Total Protein
Extracted at ionic strength 0·15	Albumins (myogen)		14
	Globulin soluble at low ionic strength (globulin X)		14 } 28
Extracted at ionic strength greater than 0·35	Structural proteins	Myosin	38
		Actin	14 } 56
		Tropomyosin	4
Insoluble in dilute NaOH or HCl	Stroma		16

The dephosphorylation of ATP is very closely linked with the release of energy for contraction.

The presence of ATP without dephosphorylation makes the contractile structure extensible.

Other substances which, like ATP, dissociate actomyosin, also make the contractile structure extensible.

In the absence of ATP or other plasticizer, the contractile structure becomes rigid and inextensible.

It is tempting to try to build these results into a theory of muscle contraction (Weber 1958, 1959). But we know very little about the mechanism of the molecular interaction between ATP and actomyosin, which appears as the fundamental process of the contraction. However, the direction in which better understanding of muscle contraction will be found is now clearer.

ACKNOWLEDGMENTS

The authors are indebted to Dr. H. E. Huxley and the publishers of *Endeavour* for permission to reproduce Figures 1 and 2, and to Professor S. V. Perry and the American Physiological Society for agreeing to the reproduction of Figure 3 from *Physiological Reviews*.

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*Based on an address to the Joint Annual Conference of the Commonwealth Cold Storage Association and the Commonwealth Associated Ice Industries, Launceston, August, 1958.**

Gas Storage of Fruit

By D. Martin

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GAS STORAGE (controlled atmosphere or C.A. storage, as it is called in North America) is now extensively practised in England and U.S.A., and is of growing importance in Western Europe and Canada. It is used mainly for apples—pears occupy only a minute proportion of the available space.

In the State of New York the C.A. apple trade has become large enough to be protected against attempts by unscrupulous dealers to pass ordinary cool-stored fruit as C.A.-stored, and against store owners who give an inadequate treatment. C.A. stores are registered, tests of gas concentration must be made daily, and complete records kept, and the desired gas concentrations must be reached within 20 days of sealing the chamber. When marketed, C.A. fruit is accompanied by a special invoice; the boxes are specially branded, and care is taken to see that they are not used again for other fruit.

HISTORY OF DEVELOPMENT

In all countries except England development has been mainly in the post-war period. The method was put forward in England in 1927 by Kidd and West, and by 1936 storage capacity had risen to 1 million boxes. Existing storages (for over 5 million boxes) are used mainly for Cox's Orange Pippin and Bramley's Seedling, and for small quantities of other varieties, such as Laxton's Superb.

In Holland and Denmark, where fruit growing for the English and German markets is expanding rapidly, many local varieties are C.A. stored, but there is a tendency to concentrate on the more popular Golden Delicious. The present capacity of Dutch C.A. stores is 11,500 tons, an increase of 100 per cent. since 1955. When Italy, which is a major fruit grower, comes more into the picture, there is likely to be a big increase in supplies of good-quality out-of-season apples in Western Europe.

The development in U.S.A. is especially interesting, as a similar pattern may be followed in Australia. Research workers in America became interested in the process soon after its discovery, and did much experimental work which allowed the industry to make a sound start. The industry developed slowly, but through the persistence of the chief worker in the field (Professor R. M. Smock, Professor of Pomology, Agricultural Experiment Station, Cornell University, N.Y.) cool-store operators in New York State became interested. The method promised to help solve the storage problems of the rather delicate McIntosh variety, the main one grown in New York State.

In 1947, when I first passed through U.S.A., Professor Smock was rather pessimistic about any large-scale interest in the process, but by 1957, when I was there again, the picture was quite different. In New York State C.A. stores had a capacity of 1 million boxes, and more stores were being erected. The method spread among the McIntosh growers across the border into Canada, and to the mid-west, and even into the major apple-growing areas of the far north-west of the United States.

The reason was that for the period 1944–1951 the average differential in favour of C.A. McIntosh had been 9*s.* per box, and for 1951–55 13*s.* 6*d.* For red Delicious for 1952–55 it had been 18*s.* To the grower storing in a public C.A. store, receipts less cost had been 9*s.* for C.A.-stored fruit and 2*s.* for regular cool-stored fruit.

In Australia scientific research has been going on since 1935, and a large volume of useful data has been accumulated. What is lacking is commercial experience. There has been little commercial development—one

* See also *Refrigeration Journal* **12**: 25–29 (1959).

store each in Tasmania, Victoria, New South Wales, and Queensland.

THEORY OF OPERATION

The aim of all cool storage is to keep the product in good condition for as long as possible. If a product (e.g. meat) is dead it has no natural resistance to the attacks of microorganisms, and at ordinary temperatures it is quickly destroyed by them. But the organisms themselves are living and their life processes are slowed down by lowering the temperature—hence refrigeration preserves a dead product by slowing down the attacks of living organisms. Refrigeration also slows the rate of chemical changes in the material itself. The lower the temperature, the greater the degree of preservation, and because the product is dead it suffers little harm from quite low temperatures.

The preservation of living things such as fruit is quite different. Because fruit is alive, it has, when young, a high natural resistance to organisms. This natural resistance declines rapidly with age, and the main effect of storage is to slow down the rate of aging, because all life processes are slowed down by lowering the temperature. However, because fruit has a delicate cellular structure and metabolic balance there are limits to the degree of cold it will tolerate. Most fruits freeze below 29°F, and are quickly killed by freezing, but many also are slowly damaged at temperatures well above their freezing point. Cool storage of fruits is therefore a compromise between keeping the temperature as low as possible to retard the aging process and keeping it high enough to prevent cold injury.

But life processes, and therefore aging, can be retarded by factors other than low temperature. All fruits respire—they take in oxygen and give off carbon dioxide. Air contains 21 per cent. oxygen, and if we lower this to 3–5 per cent. while the fruit is under refrigeration, we can slow up those life processes which use oxygen, and retard aging further. Carbon dioxide is a mild anaesthetic, and if we allow this to accumulate to 3–5 per cent. we slow down living and aging still further.

In gas (C.A.) storage the rates of the living processes are controlled by the three factors —temperature, oxygen level, and carbon dioxide level.

The control of oxygen and carbon dioxide levels depends on:

- Making the room gas tight
- The fact that fruit takes in oxygen and gives off carbon dioxide in about equal volumes
- The fact that air contains 21 per cent. oxygen and virtually no carbon dioxide.

If fruit is placed in a gas-tight room it uses up oxygen and gives off carbon dioxide. The proportions of two gases change thus:

	Carbon dioxide (%)	Oxygen (%)	
	0	21	
	1	20	
Rising	2	19	Falling
	3	18	
	4	17	
	5	16	

It is inadvisable to raise the carbon dioxide above 5 per cent., and this value may be maintained by letting out some of the 5:16 mixture and letting in outside air as the carbon dioxide tends to rise higher. This 5:16 mixture is quite satisfactory for some fruits, but not for others where a lower level of oxygen gives better results.

In order to reduce the oxygen level, the mixture is first permitted to become carbon dioxide 6 per cent.: oxygen 15 per cent. The carbon dioxide is then reduced to 5 per cent. by using a scrubber (see p. 69), making the mixture carbon dioxide 5 per cent.: oxygen 15 per cent. The pressure in the chamber is thus lowered, but the carbon dioxide is then replaced by 1 per cent. of outside air. The oxygen level then increases to 15·21 per cent., giving a net reduction from 16 per cent. of 0·79 per cent. The oxygen level is thus reduced each time this procedure is carried out, so that the composition of the mixture inside the chamber becomes progressively:

Carbon dioxide (%)	Oxygen (%)	
5	16	
5	15·21	Falling
5	14·42	
5	13·63	

It is clear that this technique affords a means of obtaining any mixture of carbon dioxide and oxygen desired.

ENGINEERING REQUIREMENTS

The first engineering requirement is a well-piped direct-expansion or brine-coil room with sufficient coil length to allow a high refrigerant temperature, for humidity must be high and deposition of ice kept to a minimum.

Secondly, the temperature distribution in the room must be very even; the variation should not be more than 1°F.

Thirdly, the exterior vapour seal must be near perfect, or water will condense on the inner gas seal and saturate the insulation.

Fourthly, the inner gas seal must be extremely good. A 2000-box room should hold a 1-in. water pressure for more than 60 min, and a 10,000-box room for more than 30 min. This is done by lining the interior walls with sheet-metal, or if the store is a masonry one, with bituminous compound trowelled on and finished with a plastic paint. There are cheaper methods, but their durability under rough treatment or for long periods is doubtful.

Fifthly, a gas analysis apparatus is required. One good, yet cheap, type is the Orsat apparatus, which must be operated with patience and common sense. Simpler, automatic, but effective types, such as the Elliott, are more expensive, but are suitable for bigger stores.

Sixthly, a scrubber is required to obtain low oxygen atmospheres. However, if the store is to handle only varieties of apples such as Democrat, Crofton, or Yates', atmospheres of the order of 5 carbon dioxide: 16 oxygen, obtained by ventilation alone, are satisfactory.

There are numerous types of scrubbers. Those using a caustic soda solution have certain disadvantages—they are tedious to handle, may be dangerous, and pose a waste-disposal problem. Promising alternative methods for removal of excess carbon dioxide, which are being developed in North America, are the use of plain water in jet or tower scrubbers and the passing of storage air over dry hydrated lime. The use of plain water depends on the fact that carbon dioxide is more soluble in cold than in warm water, so that carbon dioxide taken up by cold water inside the storage room can be released from the water outside the room. Trials are in progress with both types of scrubbers in Tasmania, and dry lime has been used successfully in experiments in New South Wales.

COSTS

It is very difficult to give an accurate figure on costs. It is naturally much higher per box for a small than a large chamber, and it is more expensive to convert an existing room than to build a new one for controlled atmosphere storage. Capital costs are between 5*s*. and 10*s*. per box above those of an ordinary cool store. Running costs are from 3*d*. per box per season for a simple ventilated store up to 1*s*. per box above ordinary cool storage charges for a scrubbed store.

In 1956 in New York State public C.A. stores were charging 6*s*. per box for scrubbed C.A. storage and 3*s*. for ordinary cool storage.

ADVANTAGES

- Storage life is increased by about 25 per cent. and shelf life is longer.
- Shrivelling, yellowing, softening, Jonathan Spot, and wastage from rots are reduced.

DISADVANTAGES

- C.A. storage is more expensive.
- The operator cannot enter the room without a gas mask or air supply.
- Only a limited number of varieties can be stored in the one room, and the room must be filled with fruit.
- More care and intelligence are required in operation.
- Some varieties are not suited to gas storage.

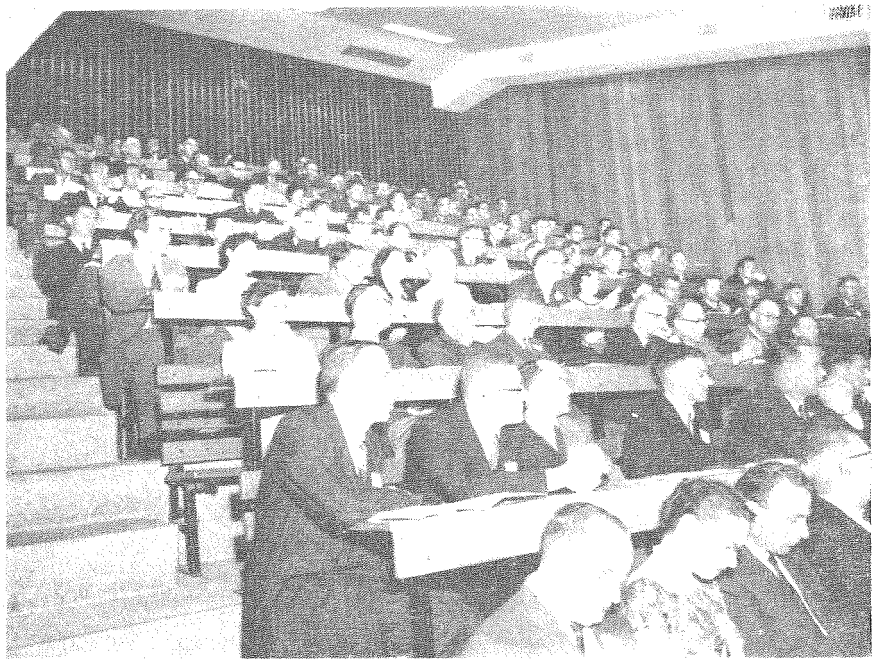
POTENTIAL IN AUSTRALIA

There is probably room for C.A. storage for up to 10 per cent. of total cool storage capacity in Australia.

Much of the overripeness and wastage in fruit on the Sydney and Brisbane markets could be prevented by C.A. storage.

I feel that C.A. storage should be approached, not with the idea of squeezing the last drop of storage life out of the fruit, but to hold fruit for a reasonable time to prevent the present high losses, and to give the consumer a better product.

A final word of warning—C.A. storage will not work miracles on fruit which has no hope in cool storage anyway. If an operator is not prepared to use common sense in selecting fruit for storage and great care in the working of a C.A. store, he should refrain from using the method.



Delegates at the Opening Session.

Conf on Food

DURING 1959, the C.S.I.R.O. Division of Food Preservation sought the opinions of representative technical people in the food industry on the desirability of holding a conference on food science and technology at a research level to encourage the sharing of knowledge and experience between investigators in industry, teaching institutions, and government organizations. The responses indicated that there would be widespread support for a research conference. Accordingly, preparations were made and came to fruition in a conference on "Control of Food Quality" which was held in the New Chemistry School, University of Sydney, November 4–6, 1959. While it was considered desirable for the conference to have a unifying theme, a broad one was chosen, and the papers presented were grouped in three symposia on the subjects: Colour Stability in Foods, Flavour Stability in Foods, and Microbial Stability in Foods.

The conference was opened on Wednesday afternoon, November 4, by Dr. F. W. G. White, Chairman of C.S.I.R.O., and the opening session was chaired by Dr. J. R. Vickery, Chief of the Division of Food Preservation.

Dr. White spoke of the vital importance of food science for the political and economic welfare of the nations of the world. He said that relaxation of international tensions depended largely on improvements in standards of living in many under-developed countries and that food scientists had unique opportunities through the Colombo Plan and the United Nations Organization to work towards this objective. Dr. White then made the following points concerning the Australian food industry:

- Within Australia, increases in population and particular problems of climate and soil make necessary an increasing emphasis on the food sciences. More knowledge is needed of the chemical, physical, and biochemical changes going on in food during harvesting, handling, transport, and treatment. Australia's dependence on healthy export markets for processed foods increases this need. Only with sound basic technical knowledge can problems arising in export and home consumption be dealt with confidently.
- The fact that the food industry and the Commonwealth Government appreciate the need for research in food science is demonstrated by the creation of the Bread Research Institute, the Wine Research Institute, and the Dairy Products Research Trust, and also in the establishment of extensive new facilities for the C.S.I.R.O. Division of Food

ference

d Quality

By J. F. Kefford
Division of Food Preservation and Transport, C.S.I.R.O.
Homebush, N.S.W.

Preservation at North Ryde.

• In the future the food industries themselves must accept greater responsibilities in industrial research, if they are to progress and prosper.

COLOUR STABILITY IN FOODS

The symposium on colour stability in foods commenced with a paper on the stability of colour in tomato products by Mr. R. A. Edwards and Professor F. H. Reuter of the Department of Food Technology, University of New South Wales. In the conventional process for the manufacture of tomato paste by vacuum evaporation of tomato pulp some deterioration in colour occurs. A novel method was described for the production of highly concentrated tomato paste in which the quality characteristics of colour, consistency, and vitamin C content are retained to a high degree. Tomato pulp is separated centrifugally into two fractions: the suspended solids, and the liquid serum. The serum is vacuum concentrated to a high solids content and then restored to the pulp to form a concentrated paste. From the subsequent discussion it appeared that the greatest practical difficulty in commercial application of this process was likely to be the continuous separation of the suspended solids from the serum so as to obtain recoveries of the order of 96 per cent. which are necessary for the process to operate at maximum efficiency.

The same authors reported on the carotenoid pigments present in Australian varieties of tomato. They demonstrated the quantitative and qualitative changes which occur in the principal pigments as tomatoes advance through successive stages of maturity defined in terms of visual colour.

Dr. B. V. Chandler of the C.S.I.R.O. Division of Food Preservation discussed leucoanthocyanidins as factors in food quality. These colourless compounds occur widely throughout the plant kingdom and their presence influences the flavour and texture of many foods. In processed foods they are responsible for colour changes that occur during heat treatment, leading to discolorations which are red in acid packs and brown at higher pH levels. Pink discoloration in canned pears is an example of this type of colour change. Field studies have shown that pears having high soluble-solids content tend to contain more leucoanthocyanidins and hence are more susceptible to pink discoloration.

In a paper on the chemistry of non-enzymic browning Dr. T. M. Reynolds, Dr. E. F. L. J. Anet, and Dr. D. L. Ingles of the C.S.I.R.O. Division of Food Preservation summarized

investigations which have elucidated some of the complex chemical reactions leading to discoloration in concentrated or dried foods. Freeze-dried fruit purées and related model systems were used to study the chemical changes accompanying browning, which is caused mainly by reactions between amino acids, organic acids, and sugars. The part played by amino acids and organic acids has been clarified, and the isolation of difructose amino acids has led to the recognition of a new mechanism by which glucose can be degraded and browning accelerated. The reactions of bisulphites with sugars were also studied, since sulphur dioxide is used to inhibit non-enzymic browning.

The absorption and retention of sulphur dioxide by fruits for drying was discussed by Mr. D. McBean of the C.S.I.R.O. Division of Food Preservation. The uptake of sulphur dioxide by fruit tissue is markedly affected by the type of fruit and by the size of the fruit pieces. The skin acts as a pronounced barrier to the ingress of sulphur dioxide to the underlying tissue. The most important processing variables affecting absorption of the preservative are the concentration of sulphur dioxide in the air to which the food is exposed and the time of exposure. Factors which increase drying rate also increase sulphur dioxide retention, presumably because during drying pathways of escape for the gas are gradually sealed. A short steam blanch or water spray after sulphuring results in increased retention of sulphur dioxide.

When the stability of the natural colouring matters in foods is unsatisfactory it is sometimes desirable to colour the foods with artificial dyes. It is the responsibility of Public Health authorities to determine which dyestuffs may be safely added to foods. However, the information concerning the biological properties, metabolism, and excretion of dyes used in foodstuffs is scanty and incomplete. A paper on some aspects of the metabolism of food dyes by Prof. S. E. Wright and Mr. N. Broadhurst of the Department of Pharmacy, University of Sydney, was therefore a valuable and timely contribution. From a study of the biliary excretion of a number of azo dyes in rats it is hoped to devise a screening test which might be used to evaluate the safety of food dyes.

FLAVOUR STABILITY IN FOODS

The symposium on flavour stability in foods, under the chairmanship of Mr. N. W. Rodd of Unilever (Australia) Pty. Ltd., was opened on Thursday morning, November 5, by Mr. D. A. Forss of the C.S.I.R.O. Dairy Research Section, with a paper on oxidized flavours in butterfat. Mr. Forss emphasized the value of modern techniques in the study of flavour changes in foods. In particular he described the use of gas chromatography and automatic recording spectrometers, e.g. ultraviolet, visible, infra-red, and mass spectrometers. A study of the addition of nordihydroguaiaretic acid (NDGA) to butterfat showed that it was a very effective antioxidant but that in the presence of acids a "fish-oil" flavour occurred which was intensified by copper and influenced by both temperature of storage and oxygen tension.

The second paper in this symposium also related to the stability of fats. Mr. W. B. S. Bishop, Mr. P. H. Brady, and Mr. J. F. Williams of William Arnott Pty. Ltd., Homebush, reported on oxidative changes in biscuits during shelf life. The stability of fats extracted from biscuits at various stages during manufacture and subsequent storage was assessed by the active oxygen method. It was demonstrated that the higher the quality of the shortening used in manufacture the longer the shelf life of the biscuits and the greater their acceptability at all stages of examination. In the active discussion which followed, it was revealed that biscuit manufacturers in Great Britain and the U.S.A. have available fats of much greater stability than those available at present to Australian manufacturers.

A paper on variation of flavour of meat by Mr. A. Howard of the C.S.I.R.O. Division of Food Preservation, Brisbane Branch Laboratory, was concerned with flavour changes in a complex high-moisture system containing proteins and fats together with minor constituents such as carbohydrates, other nitrogen compounds, and salts. Little information is available on the relation between flavour changes and changes in chemical composition, as meat passes through the post-mortem processes of development and resolution of rigor, and subsequent aging or ripening. During these processes nucleotide breakdown occurs with initial production of

inosinic acid and ultimate production of hypoxanthine. Evidence was presented that the production of hypoxanthine is a reasonable yardstick for some of the post-mortem changes in flavour and texture.

From meat the subject of discussion moved to green peas. The chemical reactions involved in the transition from a sweet succulent pea to a seed of high starch content were discussed by Dr. R. N. Robertson and Dr. J. F. Turner of the C.S.I.R.O. Division of Food Preservation in a paper on changes in sugars and starch during the development of pea seeds. Studies of the growth of pea seeds in the field showed that sugars increased initially, but subsequently the sucrose content decreased while the starch content increased rapidly. Over most of the period of development the rate of starch synthesis was proportional to the activity of starch phosphorylase which forms starch by the transfer of glucose residues from glucose–1–phosphate to a carbohydrate acceptor. The effect of environmental conditions after flowering on the carbohydrate composition of peas was examined in plants grown in the phytotron at the California Institute of Technology. Differences in temperature produced the most spectacular results. In peas grown at low temperatures starch formation was delayed and the sugar content was markedly greater than in those grown at high temperatures. Experiments of this type may be useful in determining the best environments for the commercial growing of peas for best flavour and texture. The reversibility of the enzymic reactions linking starch synthesis and sucrose synthesis interested several speakers in the discussion. At present no practicable method is known for reversing these reactions in the plant so as to convert starch into sugar. It is not unlikely, however, that field treatments could be discovered which might inhibit the conversion of the sugars to starch.

The flavour and texture of canned peas are influenced not only by the composition of the raw product but also by processing treatments. A paper by Messrs. L. J. Lynch, R. S. Mitchell, and D. J. Casimir, of the C.S.I.R.O. Division of Food Preservation, discussed effects of blanching on flavour and texture of peas. Peas for canning are commonly blanched in hot water for approximately 3 min at 200°F. This treatment has been shown to influence flavour adversely even though the canned product receives a subsequent heat treatment. Blanching for 30 sec accomplished approximately 75 per cent. of

A Group of Delegates. Left to right: G. Parker (M.B.T. Research Laboratory), Captain J. Fairbrother (Australian Military Forces), D. W. Grover (N.S.W. Dept. of Technical Education), R. A. Edwards (University of N.S.W.), W. A. Empey (C.S.I.R.O.).

A Group of Delegates. Left to right: L. Muller (C.S.I.R.O.), J. McPhillips (Sydney University), Miss E. M. Christie (C.S.I.R.O.), D. A. Forss (C.S.I.R.O.), B. Barlow (Unilever (Aust.)).

the total change which occurred in a 3-min blanch in certain measurable characteristics of the peas such as gas content, specific gravity, and maturometer reading. Canned peas blanched for 30 sec were rated higher for flavour and texture than samples blanched for 3 min.

A question was asked about the "viney" off-flavour which is reported to appear when unblanched peas are canned. No such off-flavours had been detected by the taste panel in the present experiments. It was suggested that the viney flavour might be due to 2-hexenal arising from the oxidation of phospholipids, and the possibility of looking for materials responsible for off-flavours in the foam on the blancher was discussed.

MICROBIAL STABILITY IN FOODS

On Friday morning, November 6, Professor F. H. Reuter occupied the chair for the symposium on microbial stability in foods. This session opened with a paper by Mr. J. McPhillips of the Faculty of Agriculture, University of Sydney, on some factors affecting the production of acid in milk by lactic streptococci. Four factors have been found to influence acid production in milk by these organisms. There is a stimulatory effect observed in herd milks, and in milks containing added protein hydrolysate or an aqueous extract of separator sediment. Also milks with higher buffering capacity reach higher final acidities. Inhibition of acid production is sometimes due to the action of naturally occurring agglutinins and the removal of the agglutinated lactic streptococci from the bulk of the milk by rising cream. Another inhibitory effect observed in heated milk is due partly to depression of the initial pH of the milk.

In the discussion which followed, several speakers referred to the difficulty of relating laboratory tests to bulk performance when testing the keeping quality of milk or the production of acid by strains of streptococci.

The possibility of increasing the microbial stability of foods by selecting suitable combinations of readily controlled environmental factors was stressed by Dr. J. H. B. Christian and Miss Judith Waltho of the C.S.I.R.O. Division of Food Preservation in a paper on the influence of pH and temperature on the salt tolerance of some bacteria. Experiments with a food-poisoning strain of *Staphylococcus aureus* and a strain of *Pseudomonas fluorescens* from chilled meat provided quantitative data on the extent to which salt tol-

erance and pH and growth temperatures are interdependent.

Dr. W. J. Scott and Mr. D. F. Ohye of the C.S.I.R.O. Division of Food Preservation followed with a paper on some problems of microbial stability in foods packaged in flexible films. The extent to which humidity and oxygen and carbon dioxide tensions are controlled within flexible film packages may have important consequences for the microbial stability of the packaged food. The rate and extent of changes in the composition of the internal atmosphere of the package are obviously sensitive to many factors including the temperature, the type of product, the nature and extent of the microbial contamination, and the size and shape of the package. Some qualitative and quantitative effects of these factors were demonstrated by the results of experiments on comminuted meat packaged in films with different permeabilities.

CEREAL PROTEINS

The conference concluded with a paper about surface chemical studies on cereal protein presented by Dr. N. W. Tschoegl of the Bread Research Institute of Australia. Development of techniques for the spreading of gluten films on air-water and oil-water interfaces has permitted the application of mono-layer techniques to these cereal proteins which form strongly bonded coherent films possessing marked viscoelasticity. They are more stable and at the same time more compressible than films of most other proteins. Characteristic differences in film viscoelasticity were demonstrated between the proteins from the bread cereals, wheat and rye, and those from the non-bread cereals, barley and oats. This suggests that the bread-baking value of different cereals may in some way be linked with the number or distribution of charge groups along the protein chain. It is hoped that these investigations will provide some insight into the factors responsible for variations in the bread-baking value of different types of wheat flours.

In addition to attending the technical sessions, delegates to the conference took part in inspections of the laboratories of the C.S.I.R.O. Division of Food Preservation at Homebush and at the University of Sydney, and of the Department of Food Technology in the University of New South Wales at Kensington.

The new lecture theatre block in the Chemistry School of the University of Sydney provided a most attractive and comfortable venue for the conference. The lecture theatre, which was made available through the courtesy of Prof. R. J. W. Le Fevre, was well appointed and the spacious foyer opening on to the fountain courts was much used for informal discussion between sessions and during the tea breaks.

The total registrations for the conference amounted to 177 and of these 147 actually attended: 81 from the food industry, 4 from University departments, and 62 from C.S.I.R.O. As might be expected, the majority of the delegates came from New South Wales, but there were 15 from other States. This attendance at the conference was very satisfying to the organizers and demonstrated that the usefulness of a research conference in food science and technology was well appreciated.

OBITUARY

Mr. E. W. Hicks

IT IS WITH DEEP REGRET that we place on record the death of our colleague Mr. E. W. Hicks, who died unexpectedly on November 2, 1959, at the age of 52.

Mr. Hicks was a graduate in Arts and Science of the University of Melbourne, and joined the research staff of C.S.I.R.O. in 1929. He possessed an outstanding knowledge of mathematics and physics, which he applied to the solution of problems in the transport, storage, and handling of foods.

At the time of his death Mr. Hicks was the leader of the Physics Section in the Division of Food Preservation and Transport, and a Senior Principal Research Officer of the Organization.

It is proposed to commemorate the work of this distinguished officer at greater length in a future issue.

The Evaluation of Sulphur Staining in Food Cans

By D. J. Casimir and R. S. Mitchell

Division of Food Preservation and Transport, C.S.I.R.O., Homebush, N.S.W.

IT IS WELL KNOWN that the degree of sulphur staining in food cans depends on a number of factors, notably the nature of the tinplate in the can, canning procedures, and length of the period over which the food is stored in the can. It has proved difficult to evaluate the effects of these factors visually. Research workers in the Division's laboratories have devised an instrument to measure the amount of light reflected from the interior of a can under standard conditions, and have used it to measure the sulphur staining of cans used for two separate packs: fried rice and green peas.

APPARATUS

The equipment consists of a 3-watt 6-volt light globe, a selenium photocell, a Cambridge 50-ohm spot galvanometer, a 500-ohm helipot, and switch key. As shown in Figure 1 the light is mounted in a can-centering ring attached to a base. A circular light baffle concentric with the centering ring is supported on two pillars to prevent light travelling direct to the photocell, which is mounted in a cap fitting over the open end of the test can.

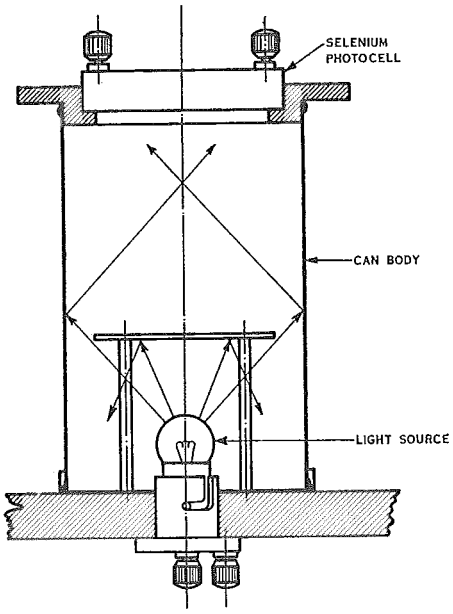
The surfaces of the base, baffle, pillars, and photocell holder are finished in a matt black. The electrical circuit is shown in Figure 2.

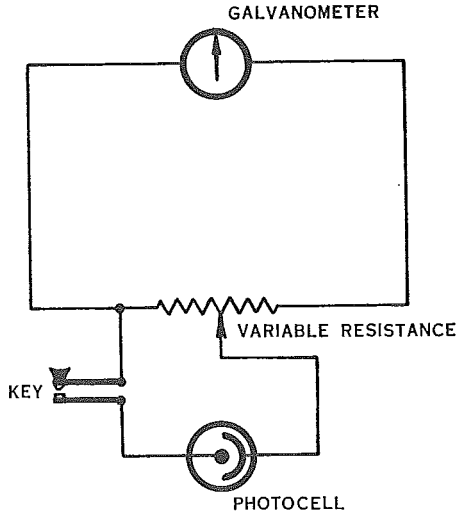
METHOD OF OPERATION

A number of unused cans, selected at random from the same batch as those to be tested, are sealed at the same time. At the end of the test period both ends of these cans are removed and the empty cylinders used to select a standard. Each cylinder is located as shown in Figure 1, and the corresponding galvanometer reading is noted. It is convenient to adjust the helipot tapping so that the galvanometer reading is around 100 scale units. A cylinder is then selected from these with a reading typical of an unstained can and the galvanometer reading is set by means of the helipot to exactly 100 scale units. Stained bodies are now substituted for the standard and the galvanometer deflection for each one is recorded as percentage reflectance. The standard body may be placed in position at any time to check that the 100 per cent. reading is correctly reproduced.

EXAMPLES OF USE

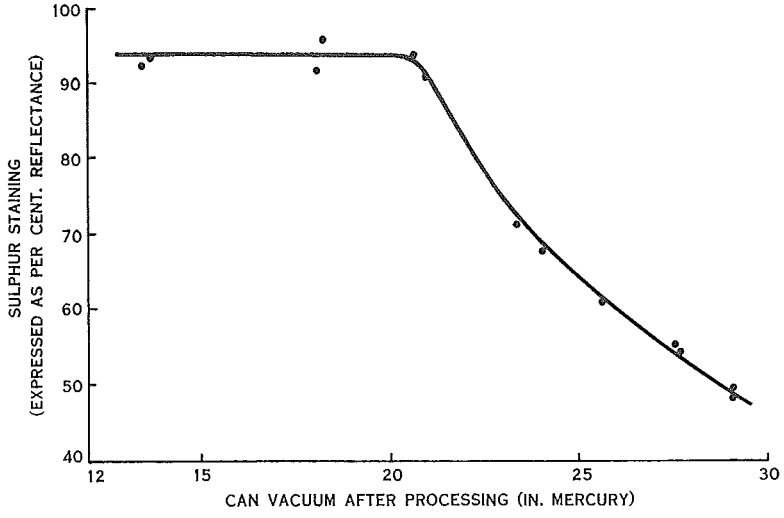
The apparatus has been used to determine the influence of can vacuum on sulphur staining in canned fried rice. The influence of can vacuum on the severity of sulphur staining when fried rice (60 per cent. moisture level) is processed for 55 min at 245°F in plain cans is shown in Figure 3. No sulphur staining





Top Left—

Fig. 1.—Apparatus for evaluation of sulphur staining in cans.



Top Right—

Fig. 2.—Electrical circuit.

Left—

Fig. 3.—Influence of can vacuum on sulphur staining in cans of fried rice.

is apparent unless the can vacuum exceeds 20 in. of mercury.

With canned peas no trend in the severity of sulphur staining could be observed with blanch times between 0 and 240 sec. However, the degree of sulphur staining was found to be related to maturity, staining being more intense with older peas. Peas with 14·9 per cent. and 9·3 per cent. alcohol-insoluble solids gave can reflectance values of 59·2 per cent. and 73·2 per cent. respectively. The cans of peas were processed for 28 min at 245°F and stored for 6 months at room temperature prior to examination.

Canned Food from Antarctica

By J. F. Kefford
Division of Food Preservation and Transport,
C.S.I.R.O., Homebush, N.S.W.

A can of golden syrup after 50 years in Antarctica.

THE C.S.I.R.O. Division of Food Preservation and Transport was recently given the opportunity to examine a can of golden syrup from Antarctica.* The can, which had come into the hands of Mr. Lionel Adams of Lysaght-Gollin Pty. Ltd., Melbourne, was part of the stores of the British Antarctic Expedition 1907–9 in the "Nimrod" commanded by Lieut. E. H. Shackleton. It was recovered from a case in the headquarters hut at Cape Royds, Ross Island, Antarctica, by Sir Raymond Priestley on February 13, 1959, given by him to Capt. J. K. Davis, and then passed on to Mr. Adams. Sir Raymond Priestley was a geologist on Shackleton's expedition and Capt. Davis was mate of the "Nimrod".

The 2-lb lever-lid can illustrated above carries a lithographed label indicating that the golden syrup was manufactured by Abram Lyle & Sons Ltd. of London. When examined on July 24, 1959, the can showed some rust on the bottom and lid, and on the side and end seams. There was a black deposit around the edge of the lid where some seepage of syrup had occurred. Internally the tinplate surface was generally clean and bright with very slight corrosion in the headspace region.

The syrup in the can had partially crystallized so that a layer of sugar crystals occupied about one-quarter of the can. The syrup had a normal golden-brown colour and characteristic flavour, and the soluble-solids content was 78 per cent. (as sucrose, by refractometer). Analysis for dissolved metals revealed a tin content of 12 p.p.m. and an iron content of 47 p.p.m. These values would not be regarded as seriously invalidating a statement on the label which guaranteed the product against metallic contamination.

In general it might be said that the golden syrup in this can was sound and edible and the can itself was in reasonable condition after 50 years in Antarctica.

* Other accounts of canned foods recovered from Antarctica have appeared in food journals in recent years. See, for example, *Canning and Packing* (1957), **27** (317): 2.

NEWS FROM THE DIVISION OF FOOD PRESERVATION AND TRANSPORT

PERSONAL

DR. JOHN GIOVANELLI has joined the Division's Plant Physiology Unit at the University of Sydney, where he will be a member of the team engaged in research on problems of plant metabolism. Dr. Giovanelli graduated in Agriculture at the University of Sydney in 1953 with first class honours in plant physiology. Soon after, he accepted an appointment at the University of California, at which he took his Ph.D. degree in comparative biochemistry in 1957. He was later granted a post-doctoral fellowship at the Johns Hopkins University, Baltimore, Maryland, U.S.A., where he studied the problems associated with hydrogen transfer in isolated chloroplasts. In 1959, at the conclusion of his studies in U.S.A., Dr. Giovanelli visited the United Kingdom and the Continent. He took up his appointment as a Research Officer in C.S.I.R.O. on October 1, 1959, in London.

MR. IVOR REY, formerly of the C.S.I.R.O. Division of Metrology, was appointed Divisional Engineer on September 21, 1959. Mr. Rey completed courses in mechanical and electrical engineering in the United Kingdom, and since coming to Australia he has taken out other professional qualifications in mechanical engineering. Mr. Rey will be responsible for the design of laboratory equipment, and will be in charge of the workshops and engineering services of the Division.

PUBLICATIONS BY STAFF

Air-dried Mutton Slices. A. R. Prater and G. G. Coote. *C.S.I.R.O. Aust. Div. Food Pres. Transp. Tech. Pap.* No. 11 (1959).

Because dried mutton mince cannot be used for a great variety of dishes the drying of mutton in slices of different thicknesses was investigated. Methods of slicing, drying, and packing are described in this paper.

The storage life of the slices was shorter than that of dried mince both as loose gas packs and compressed packs, some parts of the carcass were unsuitable for slicing, and variations in thickness and fat content affected the drying rate. Only thin slices could be dried in a reasonable time. These disadvantages, difficulties in handling and reconstituting slices, their fragility and low packing density make it unlikely that large-scale production of slices will be undertaken in place of dried mince.

Uridine Diphosphoglucose in Banana Fruit. K. S. Rowan. *Biochim. Biophys. Acta* **34**: 270–1 (1959).

In banana fruits sucrose is formed after harvest, during ripening. Uridine diphosphoglucose, which is known to be an intermediate in the synthesis of this sugar by higher plants, was isolated by column chromatography from bananas before ripening and its identification was confirmed by paper chromatography.

Water Permeability of Cells of *Chara australis* R.Br. J. Dainty* and A. B. Hope. *Aust. J. Biol. Sci.* **12**: 136–45 (1959).

* University of Edinburgh.

Chemistry of Non-enzymic Browning. III. Effect of Bisulphite, Phosphate, and Malate on the Reaction of Glycine and Glucose. T. M. Reynolds. *Aust. J. Chem.* **12**: 265–74 (1959).

It is usually considered that non-enzymic browning in foodstuffs is initiated by the reaction of amino compounds with reducing sugars, and products of the reaction between amino acids and glucose have been shown, in an earlier paper in this series, to be present in browned freeze-dried fruit. This browning in dried fruit is delayed by the addition of sulphur dioxide or bisulphites, but the experiments described here showed that the reaction between glycine and glucose was not affected by the presence of sodium bisulphite, and that its rate was increased by the organic acids in the fruits.

VI. The Reaction of Aldoses with Amine Bisulphites. D. L. Ingles. *Aust. J. Chem.* **12**: 275–9 (1959).

VIII. The Hydrolytic Reactions of Aldose Bisulphite Addition Compounds. D. L. Ingles. *Aust. J. Chem.* **12**: 288–95 (1959).

These papers describe studies on compounds which may be formed when bisulphites are present in materials containing sugars and amino acids.

VII. Crystalline Di-D-fructose-glycine and Some Related Compounds. E. F. L. J. Anet. *Aust. J. Chem.* **12**: 280–7 (1959).

X. Difructose-amino Acids as Intermediates in Browning Reactions. E. F. L. J. Anet. *Aust. J. Chem.* **12**: 491–6 (1959).

These two papers discuss the compounds formed and their effects on browning.

IX. Studies of Sugar Mono-esters of Malic Acid Found in Browning Freeze-Dried Apricots. D. L. Ingles and T. M. Reynolds. *Aust. J. Chem.* **12**: 483–90 (1959).

This paper describes work on the role of organic acids in browning of dried fruits.

Separation of Anthocyanins from Plant Extracts. B. V. Chandler and T. Swain*. *Nature* **183**: 989 (1959).

This note describes the use of powdered nylon columns to remove flavonoids from plant extracts prior to separation of anthocyanins by cellulose column chromatography.

Electric Potential Differences and the Donnan Equilibrium in Plant Tissues. G. E. Briggs† and A. B. Hope. *J. Exp. Bot.* **9**: 365–71 (1958).

Lysis of *Vibrio costicolus* by Osmotic Shock. J. H. B. Christian and M. Ingram*. *J. Gen. Microbiol.* **20**: 32–42 (1959).

Freezing Points of Bacterial Cells in Relation to Halophilism. J. H. B. Christian and M. Ingram*. *J. Gen. Microbiol.* **20**: 27–31 (1959).

Phosphorylated Compounds in Plants. II. The Estimation of Hexose Phosphates and Adenosine Pyrophosphates in Plant Tissue by the Method of Slater. K. S. Rowan. *J. Exp. Bot.* **9**: 436–45 (1958).

* Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge and Department of Scientific and Industrial Research.

† Botany School, University of Cambridge.

Copies of papers mentioned above may be obtained from the Librarian, Division of Food Preservation and Transport, Private Bag, P.O., Homebush, N.S.W. (Telephone: UM 8431, UM 6782, UM 8938).