24 June 1966, Volume 152, Number 3730 SCIENCE

Leaf Protein as a Human Food

Leaf protein, known to be nutritionally adequate, now awaits efficient manufacture and wide acceptance.

N. W. Pirie

In 1770, Guillaume François Rouelle, who had been one of Lavoisier's teachers and who figures prominently in the histories of chemistry, died. His younger brother, Hilaire Marin Rouelle, succeeded him in the post of demonstrator in chemistry at the Jardin du Roi (now the Jardin des Plantes) in Paris. H. M. Rouelle lacked the flamboyance of his brother; he is described as neat, tactful, and a good analyst. If mentioned at all in the histories, it is as the discoverer of formic acid in ants. But, in 1773, he published two interesting papers (*1*) on the composition of leaves. His brother had already classified the green material, not yet called chlorophyll, among the resins because of its solubility in lipid solvents; the new observations suggested a relationship between the residue and gluten. The credit for discovering gluten and demonstrating its resemblance to meat and other animal products is generally given to Beccari. But the sticky mass that remains when starch is washed out of flour was well known in the kitchen; it did not have to be discovered. Its similarity to meat must have been recognized centuries earlier in China, for the Chinese name for gluten, *mien chin*, means literally the muscle of wheat. Beccari called the attention of chemists to the resemblance, but Rouelle extended the category more widely by calling the material he had made "*la matière glutineuse ou végéto-animale.*" This was 65 years before the proteins were defined as a category by Berzelius.

Rouelle studied several leaf species, said that they behaved similarly, and gave details of an experiment with hemlock. He pounded the leaves to a pulp in a marble mortar with a wooden pestle, pressed out the juice through cloth, heated the juice until he could no longer keep a finger in it (Fahrenheit developed the idea of a temperature scale in 1724 but it had not yet spread widely), and filtered it through cloth again. The green coagulum remained on the cloth, and the clear liquor yielded a white or pale green precipitate on further heating. During the following century the foundations of our knowledge of proteins were laid by studies on egg white, milk, blood, and various seeds, especially those of the legumes. The presence of protein in leaves was fully recognized by those concerned with animal nutrition, but very little attention was paid to ways in which it could be extracted. Winterstein (*2*) extracted protein from dried, ground leaves with dilute alkali, but sustained work did not start until 20 years later, when Osborne and Wakeman, Chibnall and Schryver, and Kiesel *et al.* (*3*) extracted protein from the fresh leaves of several species. Since then there has been a steady increase in the amount of work done in laboratories on leaf proteins; most of it is incidental to the study of photosynthesis, virus infection, or the general metabolism of the leaf. Eight or ten years ago it may still have been reasonable (*4*) to review leaf proteins as if they were a definable category, but the scale and diversity of work has so increased that there would be little advantage in doing this now.

There has been no comparable increase in interest in the practical use of extracted leaf protein. In 1924 and 1925 K. Ereky, the Minister for Development in the Hungarian government, tried to get articles published in Britain about a method he had developed for processing green crops on a large scale. He was deliberately inexplicit about both the method and the merits of the products made, but a patent (*5*) was soon issued. The machine consisted of knives set on the opposed faces of a pair of coaxial truncated cones, and greenstuff was introduced in a stream of water which could be recycled to prevent too great dilution of the extract. Ereky did not describe how he separated the fibrous residue from the protein-containing extract, and he had some confused and largely erroneous ideas about the changes that took place in forage during autolysis and drying. He was, however, clear about the objective: to make from a fodder suitable only for ruminants a protein concentrate suitable for nonruminants. The advantages of thus partially bypassing the ruminant were stressed by Slade, and he and Birkinshaw obtained a patent on a process by which leaf protein was precipitated from weakly acidic solution, although the method had been used by Winterstein and most other workers with leaf extracts (*6*). Slade and Birkinshaw described extractions in the laboratory only, but Goodall (*7*) took out a patent for the use of full-sized sugarcane rolls for extracting juice from leaves so that it could be evaporated and used as a vitamin preparation.

By 1939, therefore, the position had been fully defined; the quantity and extractability of protein in a few leaves was known, the advantages of extracting it were recognized, some methods for

The author is head of the Biochemistry Department, Rothamsted Experimental Station, Harpenden, Hertfordshire, Britain.

doing this were known, and so were the bulk properties of the protein. All that remained was to find out whether the production of leaf protein in bulk was practicable and whether the protein would prove to be as useful as theory suggested. I started making plant virus preparations in 1934 and so gained experience in handling the normal proteins of the leaf. On the outbreak of war in 1939, many meetings were held in laboratories in Cambridge and elsewhere to discuss the ways in which scientific skill could be best used. Those of us who were interested in nutrition, remembering the effects of blockade in the 1914–18 war, suggested various projects for improving food production, with particular emphasis on protein. I suggested work designed to see whether new academic knowledge now made bulk production of leaf protein feasible. Nothing happened immediately, but in the general ferment that followed the "fall of France" in June 1940, I was asked to help the Food Investigation Board and Imperial Chemical Industries in a joint study of the problem. It seemed clear that there was little need for more work in the laboratory; instead, large-scale machinery had to be found or designed.

The Principle

Fresh leaves, rubbed or comminuted so as to liberate most of the protein from the cells, yield a pulp that is easily handled in the laboratory but that is intractable in bulk. The conventional engineering approach to material like this is either to dry it so that it can be moved through the equipment on a current of air or to add water to it until the slurry will flow. Drying is expensive, and so much of the protein in leaves denatures when the leaf is dried that alkaline extraction is needed to get any of it to separate from the fiber (*2*); much of the protein in dried leaf remains unextractable. To get a slurry that will flow satisfactorily, the water content of the pulp must be increased to 95 percent or more. If the crop contains 85 percent water when harvested, this necessitates adding to the crop four or five times its wet weight of water. Adding water (or recirculated leaf extract) on that scale is troublesome and complicates the later processes of coagulation and separation of the coagulum from the liquor. Therefore, although it is usual to extract leaf protein in the laboratory by mincing or grinding leaves with added water, work during the summers of 1940 and 1941 concentrated mainly on attempts to use existing machinery to pulp freshly harvested crops without adding large amounts of water.

In principle, the arrangement used by Ereky is that of the "breaker" or Hollander used in papermaking. It can work only on a dilute slurry, and repeated passage between the knives is necessary to get adequate subdivision. The "tobacco cutter" is a refined form of chaff cutter, and in it a block of compacted leaf is fed into the path of a reciprocating knife carried in robust guides. When the machine is set to make eight or more cuts per centimeter, especially when the knife has become blunted by use, it produces a continuous flow of pulp in which cell destruction is nearly complete. But it is not a satisfactory solution to the problem, because the output is small and the machine is easily damaged by trash that may be included in the crop. The "bowl cutter" used by meat and vegetable processers is less vulnerable but will not give adequate subdivision.

The domestic meat mincer does an admirable job in the laboratory and, with soft leaves, 375-watt (half-horsepower) machines 8 centimeters in diameter will make 10 to 20 kilograms of pulp an hour. Larger mincers of the same basic pattern are unusable because they pack the charge too tightly and generate too much heat. Many types of screw expellers, designed for pressing oil out of fish or seeds, were tested and found to be unsatisfactory for the same reason. They work on a compacted mass and, with material as rough and unlubricated as most leafy crops, friction becomes excessive. Expellers are used successfully on citrus waste and they have been tried recently on leaves (*8*), but with the object of removing part of the water rather than extracting protein.

It was often difficult to persuade manufacturers of various pulping machines, tested for such a short time that an equilibrium temperature was not reached, that frictional heating was excessive. But it is obvious from the value of the mechanical equivalent of heat that, if 22,400 watts are consumed in making 1 ton per hour of pulp containing 85 percent water, the temperature of the issuing pulp will be raised by nearly 30°C. With such a power consumption, the protein would therefore be coagulated *in situ* on a hot day unless the further complication of cooling the pulper were resorted to. These considerations show that, for efficient pulping, the mass of leaf must not be allowed to become compacted inside the pulping unit.

Sugar-cane rolls, ball mills, rod mills, edge- and end-runner mills, and dough-breakers (incorporators or pfleiderers) were tried, and all proved possible methods for making a satisfactory pulp, but they were all, for various reasons, unsatisfactory. Stamping mills of the type used to disintegrate ores were not tried, because laboratory work (*9*) had not at that time demonstrated the possibilities of pulping by impact. It may be that this method deserves further study.

Hammer mills of the conventional type, in which the charge remains inside the mill until it has been comminuted sufficiently to go through holes in part of the mill casing, were tried at an early stage. All had the same defect; if the holes were small enough to ensure adequate subdivision they soon clogged with fiber. This defect could be overcome by flushing with water, but the extract seemed to us inconveniently dilute. This method has, however, had recent advocates (*10*). In discussion with William Christy (of Christy & Norris, Chelmsford) the idea evolved of making an uncloggable mill, without a screen, by mounting the beaters on a shaft so that the pulping chamber was two or three times longer than its diameter. This would be fed at one end and discharge at the other, and its operation would depend on adjusting the rate of movement from one end to the other so that pulping was adequate. A small prototype was tried, and then work was discontinued.

As I have said, work on leaf protein started in the tense period after the fall of France when it looked as if Britain would have to depend on her own efforts. As this initial tension wore off, the usual official distrust of all novel approaches reasserted itself. There was a hilarious period in which I was told, at the same time and sometimes by the same people, that the idea of making leaf protein as a human food could not possibly be practical, and that it was so important that I must be more secretive and not explain, to those whose pulpers I was testing, what was intended, lest the Germans should get hold of an important idea. The generous supply of food from the United States under Lend Lease finally made it obvious that there would be no need for British self-sufficiency in wartime.

New Extraction Equipment

Although there was no longer any official interest in or support for work on leaf protein, sufficient unofficial interest remained for the idea to be discussed at meetings, described in articles, and pursued in laboratories (*11*). One reason for this continued interest was the realization that a worldwide food shortage, affecting both Britain and the underdeveloped countries, might arise after the war. This idea gained strength in 1947.

In 1948 a grant from the Agricultural Research Council enabled large-scale work to start again. A Christy & Norris "coir sifter" was modified so that the direction of movement of the charge in it was reversed; the crop was fed in axially and the pulp discharged tangentially at the other end. The working chamber was a cylinder 1.4 meters long and 0.9 meter in diameter within which a set of fixed beaters rotated. This machine underwent continuous improvement, and a description of it after 10 years of evolution was published (*12*). Evolution has continued, though the basic principle remains the same. The most recent pulper, the seventh to be made, is shown in Figs. 1 and 2.

With soft material, such as sugar-beet tops or the waste from pea canning, this pulper consumes 15,000 watts when fed at a rate of 2 tons per hour. Tougher raw material goes through more slowly, but no crop available in Britain from which a significant amount of protein can be extracted goes through at less than 0.5 ton per hour. If a unit were being set up to process material faster than this it would probably be inefficient to use larger machines, because effective pulping goes on at the periphery only and larger machines would contain a larger proportion of uselessly spinning metal in the middle. Instead, as in the oil-seed industry, a battery of machines of the present size, or even smaller, would seem to be preferable.

Problems comparable to those encountered in pulping leaves are encountered in getting the protein-containing juice out of the pulp. From a survey (*13*) of the various possibilities, and from the tests that had been made, it seemed that the ideal press for this material would apply 1.5 to 3.0 kilograms per square centimeter to a mass of pulp not more than 2 centimeters thick initially, would maintain the pressure for 5 to 10 seconds, and would produce no relative movement between the pulp and the filtration surfaces while there was pressure on them. A machine (*14*) meeting these requirements consists of a conveyor belt, whose tension is adjustable, that presses the pulp against a perforated pulley; the juice flows through the perforations into the pulley and out over its edges. It would obviously be advantageous to have perforations in the belt also, but this has not, as yet, proved feasible.

Although the processes of pulping a mass of leaf and pressing the juice out of the pulp are dissimilar, the idea of performing both operations in one machine is attractive. Rollers and screw expellers do not extract a satisfactory percentage of the protein unless the material is passed through them several times. It is only because of their inadequacy as presses that they work as pulpers, and vice versa. If several passes are going to be needed there is no advantage in using the same machine for all of them; it is better to have an efficient pulper followed by an efficient press.

However, when simplicity is more important than maximum efficiency, as in a laboratory that is just beginning to work with leaf protein or in a relatively unsophisticated community, there are advantages in making the extract in one operation. We (*15*) therefore made a "Village Unit" in which 100- to 200-kilogram lots of leaf are pulped by a heavy, ribbed roller that is driven round on a horizontal bed. As the juice is released it is pressed through perforations in the bed. This unit consumes less power, per ton of crop handled, than the large unit. Perhaps for this reason it extracts only 40 to 50 percent of the protein, whereas the large unit extracts 50 to 70 percent of the protein from the same crop. We hope to get better extraction by changing the design of the ribs.

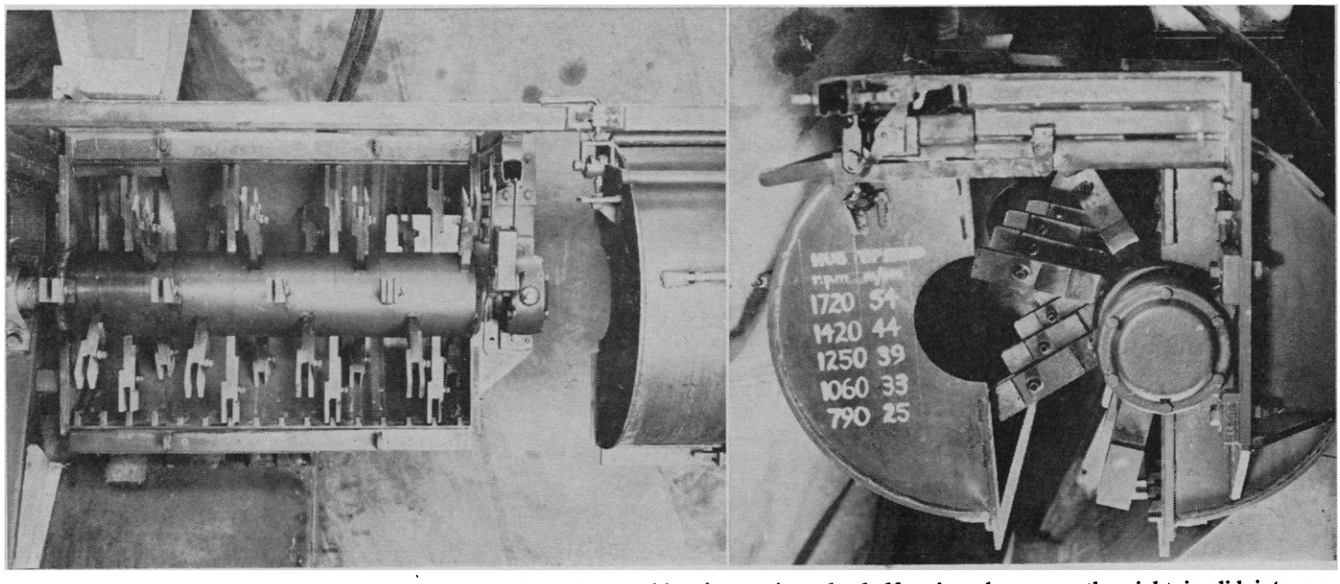

Fig. 1 (*left*). Leaf pulper opened for cleaning. When the machine is running, the half-casing shown on the right is slid into position and clamped. The crop falls down the chute (top left), moves to the right inside the drum, and is discharged as pulp through the opening. Some of the beaters carried by the rotor are wedge-shaped and so move the crop from left to right. By changing the speed and arranging a suitable ratio of plain and wedge-shaped beaters, crops of varied textures can be properly pulped. Fig. 2 (*right*). View of the half-opened leaf pulper from the discharge end. It rotates clockwise.

Properties and Uses of the Protein

Rouelle coagulated the protein in leaf extracts by heating, and this remains the most convenient method, though protein can also be precipitated with acid, with salts, or by aging. As in the making of cheese and soap, clean separation and rapid filtration depend on care over details of the technique (*16*). If the juice from most leaf species is heated quickly so that all of it reaches at least 70°C, the coagulum can be separated by standard methods of filtration or centrifugation. By washing and pressing, the protein is made into a dark green block, with the texture of cheese, containing 60 percent water; the dry matter contains 9 to 11 percent nitrogen, 20 to 25 percent lipid, 5 to 10 percent starch, and a variable amount of ash; this is often as little as 1 percent but, for reasons that are not yet clear, exceeds 10 percent on some batches. The relationship

$$\text{Protein} = \text{Nitrogen} \times 6.25$$

is probably less misleading with this material than with most other foodstuffs, because the material has been washed nearly free from water-soluble nonprotein nitrogen compounds. Lipid nitrogen is only 1 to 2 percent of the total nitrogen, and nucleic acid is so rapidly hydrolyzed by leaf ribonuclease that the amount is significant only when special precautions are taken to coagulate the juice immediately after it has been liberated (*17*).

Leaf protein made in this way is a mixture of many individual proteins. It is unlikely that all these component proteins in any species of leaf will be deficient in the same amino acid or that differences in age or nutrition (within limits compatible with harvesting a reasonable weight of crop) of one species will greatly affect the ratios in which the component proteins occur. The amino acid composition of many bulk preparations (*18*) bears out these expectations and, judged by its amino acids, leaf protein appears to be nutritionally better than the seed proteins and as good as animal proteins other than the exceptional proteins of egg and milk. This conclusion from chemical analysis is borne out by feeding experiments on pigs, rats, chickens, and infants (*19*), but preparations vary in nutritive value to an extent greater than can readily be explained by differences in the amino acid composition. This is probably the result of changes taking place during preparation or storage—through reaction of carbohydrates, phenolic compounds, or unsaturated fatty acids with amino acid residues, for example. We are trying to clarify the position and to prevent such reactions should they be the reason for the variability of our products.

The dark green color and faint spinach- or tea-like flavor give leaf protein little immediate appeal to many people. It is easier to get immediate acceptance if the material is decolorized by solvent extraction, but experience shows that most people accept the novel appearance in a few weeks; the extra process of decolorization seems therefore to be both unnecessary and wasteful. Furthermore, dark-colored and sometimes greenish foods are already part of the normal cuisine in many countries such as India, West Africa, South East Asia, and New Guinea. We have, however, devised several methods for presenting leaf protein on the table in such a way that its appearance is not obtrusive (*20*); these serve to maintain interest in the product while familarity is being won. For many years we have been eating 5- to 10-gram quantities of leaf protein and giving it to visitors and lecture audiences, encased as in ravioli, rissoles, and similar dishes. This form of presentation, though adapted to our special needs, is not wholly unrealistic, for the amount taken is about 1/10 of the protein needed in a day. But presentation is obviously a matter that must get skilled attention in every region where leaf protein may be used.

Byers and Sturrock (*21*), from measurements of protein extracted from crops grown at Rothamsted during the past few years, concluded that 1000 kilograms per hectare would be attainable in a year with average weather. The protein not extracted remains in the fiber and can be used as fodder, and the soluble leaf components have already been found useful (*22*) as substrates in industrial microbiology. Much more work is needed on the use of these by-products because, for the sake of both economy and the amenities, it will be essential to use them fully.

Desirable Future Developments

Although research has gone on, both on the laboratory and on the technological scale, for many years, the idea that leaf protein could play a significant part in alleviating the world's increasing protein shortage has not yet gained sufficient general acceptance for large-scale production to be undertaken. It should be clear from this article that the protein can be made in bulk, that it has sufficient nutritive value to be worth making, and that the product is palatable. These points have not gone unnoticed. There have been bursts of more-or-less well informed press publicity, and several instances of editorial or semiofficial commendation (*23*), but work is at present so underendowed as to make its continuance, let alone its progress, uncertain. Machines of our design have been used in Jamaica, are being used in India, and are about to be used in Uganda and New Guinea; machines in which the crop is pulped in the presence of a large amount of water are used in Israel. These developments are very valuable for maintaining interest in the project but, so far as I know, none is so well financed as to be able to carry the research so far that large-scale routine production and use can start.

The reasons for this hesitant approach are simple. Countries such as Britain and United States, where the research could easily be done, are not at present in need of new sources of protein; countries that need protein, especially those in the wet tropics, have research services that are already overstrained; the people who are most in need of new sources of protein are impoverished, so commercial interests have no incentive to embark on the necessary developmental research. No one would argue that the leaf is the only source from which the necessary protein can be got, though in some regions this seems probable. It takes its place alongside such sources as cottonseed, the legume seeds, fish, coconut protein, and microbial protein (*24*). But, if it is agreed that a prima-facie case has been made out for its potential value, an adequately endowed laboratory should be established in the wet tropics that would, among other things, see whether the potentiality can be turned into an actuality.

The first part of the research would be agronomic. We know a score of plants suitable for leaf protein production at Rothamsted, and several have been found in laboratory-scale work (*25*) in the tropics. Field work is needed to devise a farming system that will maintain a steady crop supply. At the same time the extractability of the protein in by-product leaves such as jute, sugar cane, and sweet potato, and pests such as water hyacinth, would be investigated. During the preliminary

phases of this work, it would be reasonable to use our methods without modification, and the unit at Rothamsted should continue until there is a fully functional unit elsewhere so that these methods can be demonstrated. Existing methods will certainly be improved on, but a method cannot be improved until it has been learned. Feeding trials will then be needed in which leaf protein is compared, not with proteins such as casein that are not going to be available throughout the world on an adequate scale, but with the other novel proteins that could be made locally. These comparisons should be made on several test species, the proteins should be used as part of a realistic human diet, and the tests should continue long enough to permit the physiological or microbial adaptation that follows every change in diet. It is obvious that the important thing about a novel protein source is the usefulness of the protein when eaten regularly.

A widespread 20th-century myth is that people will starve in the presence of good but unfamiliar food. It is possible to change food habits, but it takes skill and patience, as early explorers and contemporary food manufacturers well know. There will be trouble if you tell people to eat a novelty, but I have had a whole village in New Guinea clamoring for leaf protein simply by wandering round the village eating it myself. This is the essence of presentation: devise dishes acceptable to those who are doing the research on the novelty, eat it manifestly yourself, and do not expect quick results.

When thinking about the protein shortage in much of the world, it is well to remember that some regions, the United States and Australia for example, that now have embarrasing food surpluses, were initially so underdeveloped that many settlements had to be abandoned because of starvation. Workable methods for adapting traditional agriculture to the new environment were then devised. It will probably be more difficult to make the wet tropics nutritionally self-sufficient, but there is no reason to think that, with adequate research, this cannot be done.

Summary

Protein was recognized as a component of leaves 150 years before it was seriously investigated, and a further 20 years elapsed before it was tried as a human food. During the next 20 years machinery was perfected for processing fresh leaves, both at the 1-ton-per-hour rate and in 100- to 200-kilogram batches, and extracting half to three-quarters of the protein. The protein is better nutritionally than most seed proteins, as good as many animal proteins, and can be presented on the table in palatable forms. Leaf protein is probably one of the foodstuffs that will be used, especially in the wet tropics, in ameliorating the protein shortage that now exists.

References

1. H. M. Rouelle, *J. Med. Chir. Pharm.* **39**, 262 (1773); **40**, 59 (1773).
2. E. Winterstein, *Ber. Deut. Bot. Ges.* **19**, 326 (1901).
3. T. B. Osborne and A. J. Wakeman, *J. Biol. Chem.* **42**, 1 (1920); A. C. Chibnall and S. B. Schryver, *Biochem. J.* **15**, 60 (1921); A. Kiesel, A. Belozersky, P. Agatov, N. Biwschich, M. Pawlowa, *Z. Physiol. Chem.* **226**, 73 (1934).
4. N. W. Pirie, in *Modern Methods of Plant Analysis*, K. Paech and M. V. Tracey, Eds. (Berlin, Springer, 1955), vol. 4, p. 23; ———, *Ann. Rev. Plant Physiol.* **10**, 33 (1959).
5. K. Ereky, British Patent 270629 (1926).
6. R. E. Slade, *Brit. Assoc. Advan. Sci. Ann. Rep.* **1937**, 457 (1937); R. E. Slade and J. H. Birkinshaw, British Patent 511525 (1939).
7. C. Goodall, British Patent 457789 (1936).
8. T. W. Casselman, V. E. Green, Jr., R. J. Allen, Jr., F. H. Thomas, *Florida Univ. Agr. Exp. Sta. Gainesville, Tech. Bull.* No. 694 (1965).
9. N. W. Pirie, *Rothamsted Exp. Sta. Ann. Rep.* **1952**, 173 (1952).
10. I. H. Chayen, R. S. Smith, G. R. Tristram, D. Thirkell, T. Webb, *J. Sci. Food Agr.* **12**, 502 (1961) .
11. N. W. Pirie, *Chem. Ind.* **61**, 45 (1942); E. M. Crook, *Biochem. J.* **40**, 197 (1946); ——— and M. Holden, *ibid.* **43**, 181 (1948).
12. M. N. G. Davys and N. W. Pirie, *Engineering* **190**, 274 (1960).
13. N. W. Pirie, *J. Biochem. Microbiol. Technol. Eng.* **1**, 13 (1959).
14. M. N. G. Davys and N. W. Pirie, *J. Agr. Eng. Res.* **10**, 142 (1965).
15. ———, *ibid.* **8**, 70 (1963).
16. J. E. Morrison and N. W. Pirie, *J. Sci. Food Agr.* **12**, 1 (1961).
17. N. Singh, *Biochim. Biophys. Acta* **45**, 422 (1960).
18. B. P. Pleshkov and L. Fowden, *Nature* **183**, 1445 (1959); E. D. Gerloff, I. M. Lima, M. A. Stahmann, *Agr. Food Chem.* **13**, 139 (1965).
19. J. Duckworth and A. A. Woodham, *J. Sci. Food Agr.* **12**, 5 (1961); J. Duckworth, W. R. Hepburn, A. A. Woodham, *ibid.*, p. 16; J. C. Waterlow, *Brit. J. Nutr.* **16**, 531 (1962); A. A. Woodham, *Proc. Nutr. Soc. Engl. Scot.* **24**, 24 (1965); K. M. Henry and J. E. Ford, *J. Sci. Food Agr.* **16**, 425 (1965).
20. J. E. Morrison and N. W. Pirie, *Nutrition* **14**, 7 (1960); M. Byers, S. H. Green, N. W. Pirie, *ibid.* **19**, 63 (1965).
21. M. Byers and J. W. Sturrock, *J. Sci. Food Agr.* **16**, 341 (1965).
22. A. G. Jönsson, *Kungl. Lantbrukshogskols Ann.* **28**, 235 (1962).
23. New York *Times* (10 July 1955); "Possible Nonmilitary Scientific Developments and Their Potential Impact on Foreign Policy Problems of the United States" Document 45633 (U.S. Govt. Printing Office, Washington, D.C., 1959); *Med. J. Australia* **46** (27 Feb. 1960); *Indian Med. J.* (Apr. 1960).
24. N. W. Pirie, *J. Roy. Soc. Arts* **104**, 511 (1958); *J. Agr. Soc. Univ. Coll. Wales* **44**, 54 (1963); *J. Roy. Statist. Soc. Ser. A* **125**, 399 (1962); *Proc. Congr. Intern. Agric. Aliment. Zones Trop. Sub-trop., 1st, Abidjan, 1964*, Ca 4.
25. M. Byers, *J. Sci. Food Agric.* **12**, 20 (1961); N. Singh, *J. Food Sci. Technol.* **1**, 37 (1964).