In quick pursuit of microbes

If you've ever copped a bout of traveller's diarrhoea, you'll know that microbes in food can give you a hard time. But stomach upsets are really among the milder types of food poisoning; far more severe — and potentially fatal — are conditions such as botulism, which can result from eating canned food contaminated with the bacterium *Clostridium botulinum*.

As well as sometimes having the potential to cause loss of life, food spoilage also wastes money, so detecting it quickly — and while it is still in an early stage — is clearly important on two counts. At present, the commonly used methods are ones that have changed little from the days of that pioneering microbiologist Louis Pasteur. They involve taking a sample of the contaminated foodstuff and trying to culture the offending microbe or microbes in a medium suitable for their growth. Waiting for the culture to grow up on a Petri dish — in order to identify it — can take days or even weeks.

What's worse, microbes that have caused spoilage and then died will, of course, not grow to give a culture and so the food, possibly laced with a secreted bacterial toxin, will appear sterile (and therefore safe) when it is not.

But now all this may change, thanks to work carried out by Dr Michael Eyles and Mr Reg Adams at CSIRO's Division of Food Processing in Sydney. They are investigating a technique that does not involve preparing bacterial cultures: instead, it relies on detecting bacterial waste products, or metabolites. And it produces results within half an hour.

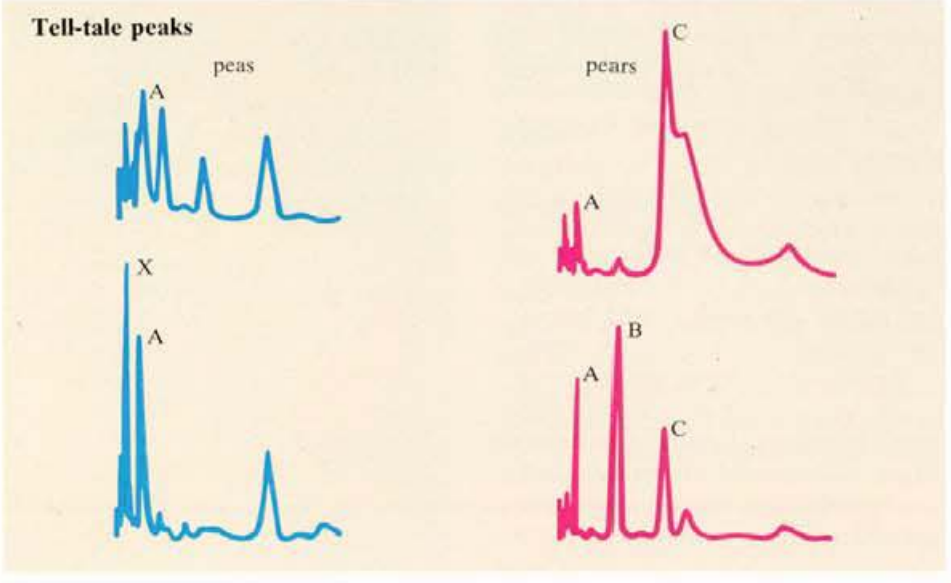
Waste products

As they grow, microbes change the chemical composition of the substance in which they live, absorbing some compounds and secreting others. Chemical analysis can detect the changes — particularly the presence of the secretory products — and, by comparison with a foodstuff known to be sterile, can tell whether microbes are, or were, growing. (It will not usually reveal the actual presence of any microbes not actively growing and hence metabolising.)

The scientists have concentrated on detecting the volatile fatty acids (fats containing from two to eight carbon atoms) and alcohols that many microbes produce. Now, most types of food represent extremely complex bits of chemistry and contain a whole host of different compounds. Trying to detect one, present in a tiny amount among all of these, may seem as futile as the proverbial quest for the needle in the haystack. But a technique well known to biochemists, namely gas chromatography, can help out.

This depends on a simple enough principle: like all types of chromatography, it relies on the fact that molecules of different substances move at different rates in a particular medium — whether it be paper or gas — and so become separated. In gas chromatography, the researchers use a device into which they inject samples. The more volatile substances travel faster through a carrier gas — in this case inert nitrogen — and so reach the other end of

The results of a gas-chromatographic separation of volatile fatty acids in canned pears (right) and peas (left). In each case, the unspoiled sample is on the top. In the pears, the bacteria have markedly increased the concentrations of compounds A (which is acetic acid) and B (*n*-butyric acid). Compound C has decreased. Equally severe changes are evident with the peas, where compound X (not yet identified) is present in large quantities in the spoiled food.



Bugs in food

Much of the food we eat contains microbes. But don't worry: we are equipped to live in a world that is full of them. Relatively few species of bacteria and fungi can harm us when we ingest them.

It is the continued growth of microbes in or on food — rather than their mere presence — that makes the food aesthetically unfit for human consumption, although not necessarily always dangerous to health. Many bacteria digest proteins to produce a number of foul-smelling substances, such as the gas hydrogen sulfide (responsible for the stink of rotten eggs). These smells warn us that bacterial activity has reached a high level, and it is wise to avoid the food in question, even if the bacteria are not technically harmful.

Several fungi produce poisonous mycotoxins, which can give us unpleasant symptoms and even be fatal. Lysergic acid, the active component of LSD, is one such, as is aflatoxin, from a fungus that may grow on damp peanuts. (See *Ecos* 49 for more on preventing the growth of aflatoxin-producing mould.)

Bacteria too can produce toxins, many of which have no smell or taste in the concentrations at which they are normally found. One of the most notorious of these comes from the bacterium *Clostridium botulinum*.

This organism, found in deep soil and occasionally animal faeces, cannot tolerate oxygen. Indeed, the oxygen in the atmosphere will poison it. But its hardy spores can survive in air. And the bacteria can live in a sealed tin of food, where there is no air.

The proteinaceous toxin that *C. botulinum* makes is one of the most poisonous substances known. As little as one- or two-millionths of a gram can kill a human. It doesn't take much calculating to realise that a few grams of the pure toxin — a spoonful — could kill every man, woman, and child in a city.

This particularly lethal biological molecule affects its victims by preventing the release of the neurotransmitter acetylcholine, which carries the signal from one nerve to another or to a muscle. The symptoms of poisoning start with visual disturbances and difficulty in swallowing, and proceed to increasingly severe paralysis, as signals to muscles fail to get through. Death comes when the respiratory muscles can no longer function.

Interestingly, the bacterium itself is not really responsible for the toxin. Although this is made in the bacterial cells, the information for its manufacture comes from a viral gene. Only those bacteria infected with the virus will be able to produce the toxin.

When a can has been contaminated with *C. botulinum* or its spores, and the bacteria have successfully grown (which will depend on the can's contents), then the toxin may well be present even if the bacteria have since died. (In this case boiling the contents may destroy the toxin, but other bacterial toxins, such as that produced by some strains of *Staphylococcus aureus*, can withstand boiling for several minutes, although this treatment kills the bacteria.)

Botulism is not an infectious disease; it is, in the literal sense of the word, an intoxication. Antibiotics are therefore not effective. The only possibility of help is a rapidly administered antitoxin that can bind to and neutralise the toxin.

The severity of botulism has meant that canning procedures are rigorously scrutinised and are now very safe. (Home-bottling is another matter.) In the last few decades, we have had no cases of botulism from food commercially canned or bottled in Australia.

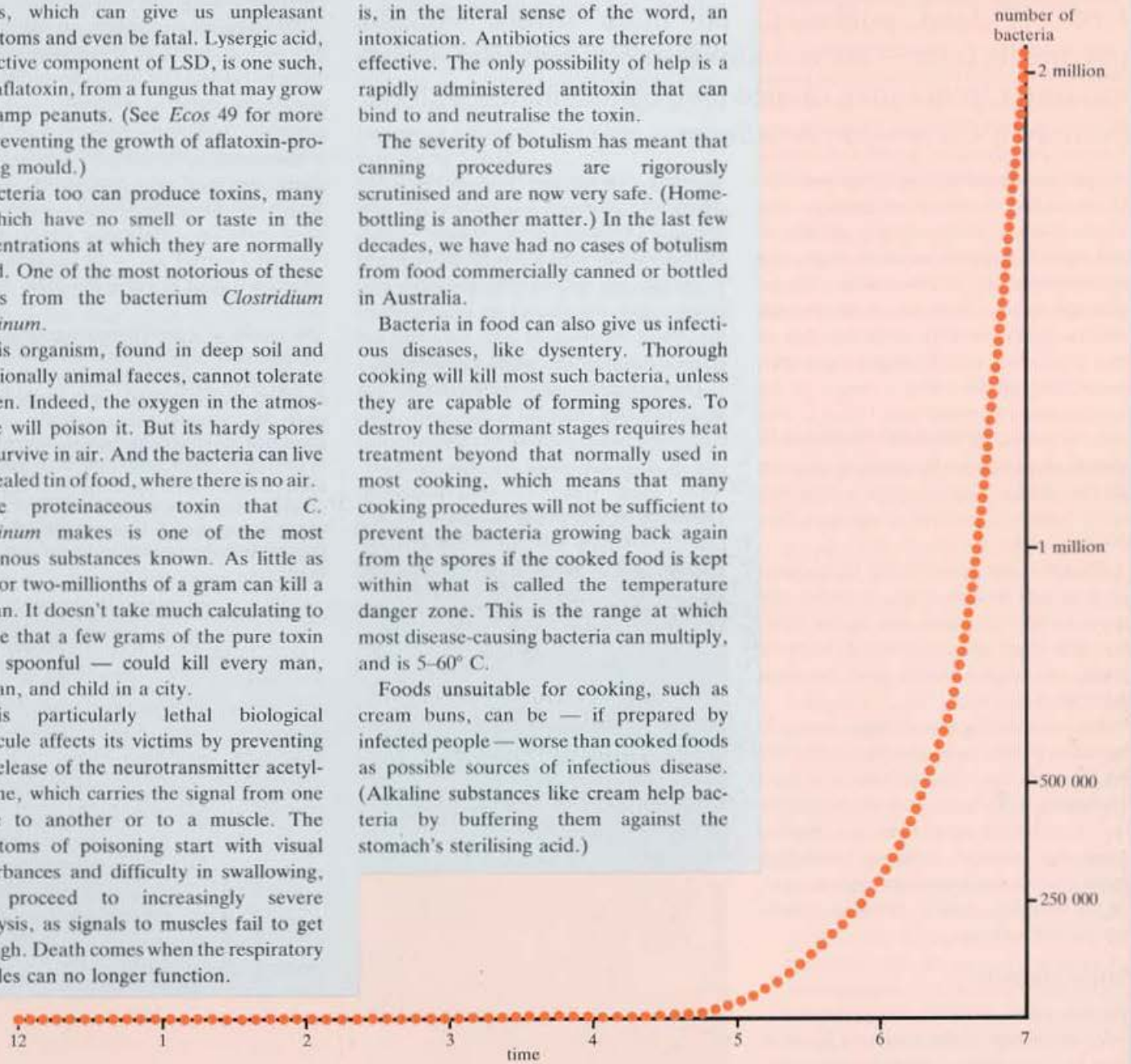
Bacteria in food can also give us infectious diseases, like dysentery. Thorough cooking will kill most such bacteria, unless they are capable of forming spores. To destroy these dormant stages requires heat treatment beyond that normally used in most cooking, which means that many cooking procedures will not be sufficient to prevent the bacteria growing back again from the spores if the cooked food is kept within what is called the temperature danger zone. This is the range at which most disease-causing bacteria can multiply, and is 5–60° C.

Foods unsuitable for cooking, such as cream buns, can be — if prepared by infected people — worse than cooked foods as possible sources of infectious disease. (Alkaline substances like cream help bacteria by buffering them against the stomach's sterilising acid.)

Naturally, workers in the food industry are trained to prevent bacterial contamination by not mixing cooked and raw foods and the utensils used for them, by minimising direct hand contact with cooked and prepared foods, and by keeping food either above or below the temperature danger zone.

Under good conditions — a warm environment and plenty of food — bacteria can divide every 20 minutes or less. One bacterium in your food, which is nothing to worry about, can become a frightening 2 million in 7 hours. Eventually, the bacteria's own waste products and even lack of food will prevent further increases.

The reality of exponential growth



the device more quickly. This is the basis of the separation.

To identify the chemicals that comprise your original mixture, you must first calibrate the device using known samples, and note the characteristics of each substance. You can then match the unknowns from your mixture with these.

The research team used the technique on a variety of spoiled products (which had been sent to the laboratory for investigation) ranging from canned asparagus and canned pears to aseptically packed meat and fruit juice. The scientists not only sampled the materials for chromatographic analysis, but also performed the traditional microbiological diagnosis by culturing any living organisms present. Of course, it was necessary to carry out a 'control' series of tests, and so they also analysed unspoiled samples of the products.

They compared the gas chromatography results — in the form of graphical printouts — from the spoiled and unspoiled goods. The differences were quite clear, although they varied with different products. For example, the profiles of the good and bad tinned asparagus varied in only one place — a peak in that of the spoiled sample did not occur in the profile of food from the 'healthy' tin. This peak represented the presence of the chemical *n*–butyric acid — a volatile fatty acid that had been produced by the metabolism of the contaminating bacteria.

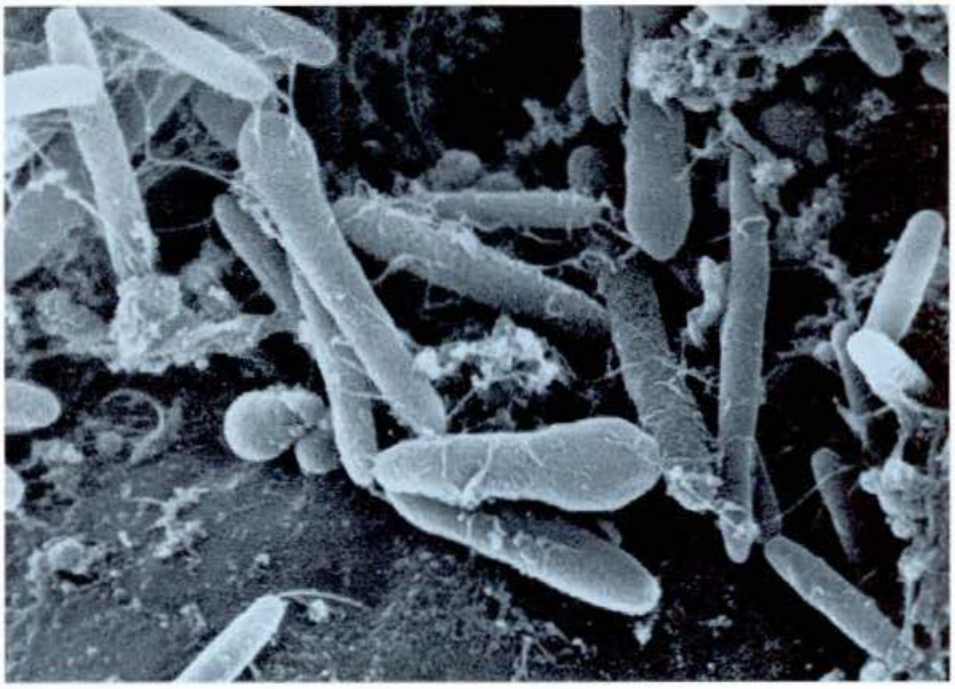
A matched pair of tinned pears told a different story. In this case, not only did the spoiled sample produce extra peaks not found in the analysis of the good food, but also some of the peaks present in that analysis were reduced or entirely absent in the spoiled food's profile. This suggests that the bacteria were consuming some of the major food components detected by the chromatography, as well as producing their own products.

Identifying the culprit

Analysis of the bacterial metabolites in food may not tell you what type of bacteria caused the spoilage. Only culturing, combined with microscopy and biochemical tests, can conclusively do this. However, the team did notice that some information about the microbes involved could be gleaned from the profile of chemical changes. And, when combined with light microscope observations of bacterial shape and size and with knowledge of the foodstuff involved (bacteria have their preferences and some will seldom grow in certain foodstuffs because of inappropriate pH or other factors), that information enabled the scientists to narrow down the likely culprits to only a few closely related species.

This result is attainable very quickly, needing only half an hour for the chromatography and a little less for the microscopy. It means that we can take action right away, knowing at least what class of microbe we are dealing with. Culturing — although slow — will still be important in revealing the precise nature of the species involved.

The clues about the culprit that the chromatographic profile gives will often allow scientists to tell at what stage, during the course of processing and packaging food, the contamination occurred.



A frightening foe: a few tiny *Clostridium botulinum* bacteria — each about one-thousandth of a millimetre wide — viewed with a scanning electron microscope.

For example, bacteria that can produce spores are heat-resistant because their spores will often survive boiling. Those that do not form spores are heat-sensitive. The scientists can differentiate between the two groups on the basis of detecting two organic compounds that only spore-formers make.

If the food in a spoiled can contains these compounds, then the contamination was caused by spore-formers and could have occurred before heat-sterilisation. On the other hand, contamination that reveals a different profile, lacking the compounds, means that the culprits are not spore-formers and could only have gained entry after the sterilising — either that, or the sterilising procedure was inadequate.

Cases of contamination in packaged foods are rare in Australia, but, to reduce the risk of disease, it behoves us to use every possible method for speedy confirmation when it does occur. *Bon appetit!*

Roger Beckmann

More about the topic

Detection of microbial metabolites by gas chromatography in the examination of spoiled canned foods and related products. M.J. Eyles and R.F. Adams. *International Journal of Food Microbiology*, 1986, **3**, 321–30.

Rapid chromatographic detection of microbial spoilage of commercially sterile foods. M.J. Eyles and R.F. Adams. *CSIRO Food Research Quarterly*, 1986, **46**, 90–4.