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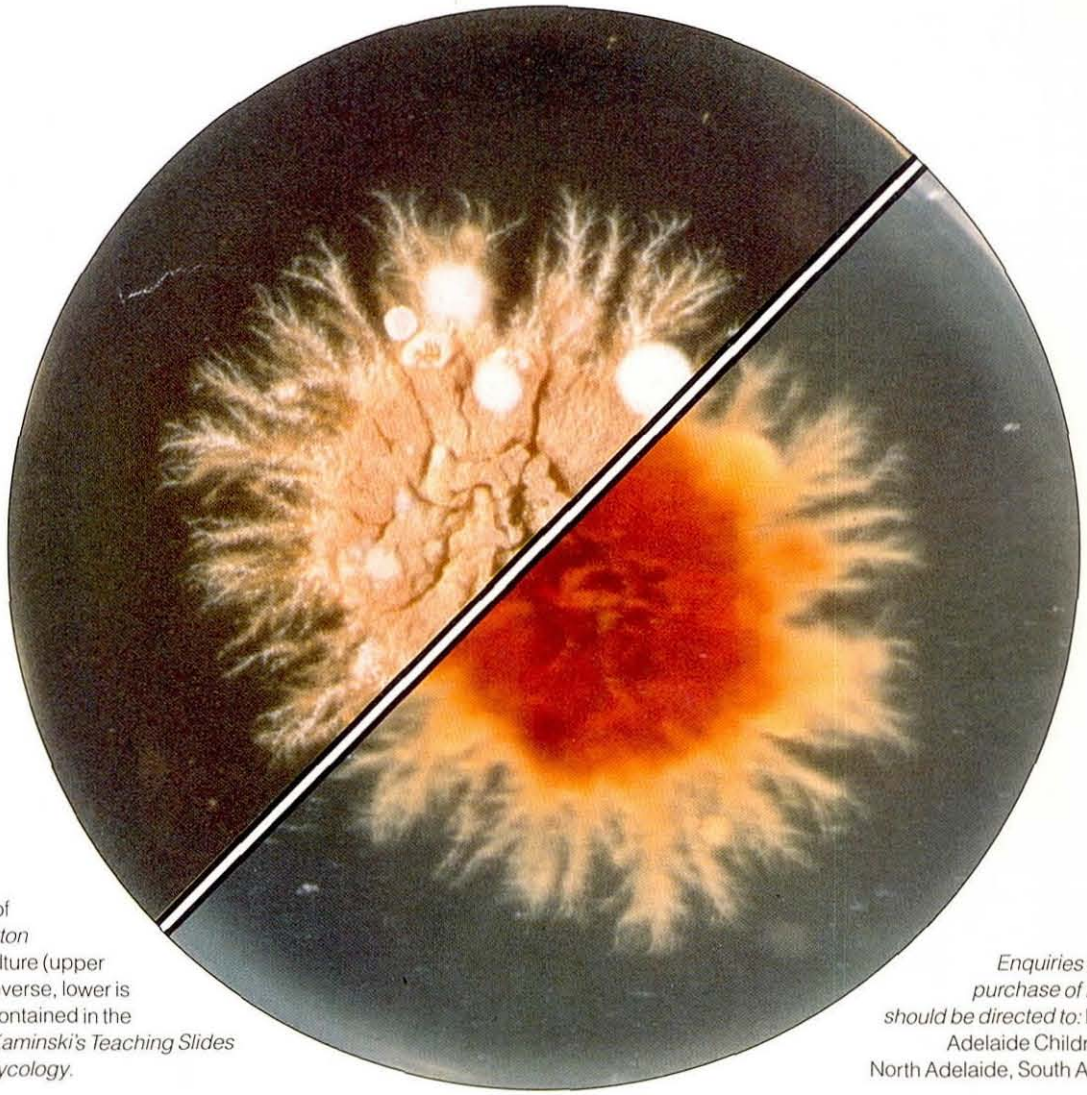

These slides of *Epidermophyton flocossum* culture (upper segment is obverse, lower is reverse) are contained in the collection of *Kaminski's Teaching Slides on Medical Mycology.*

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Microbiology of foods in modified-atmosphere packaging

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Modifying the atmosphere surrounding food products is now widely used to extend their shelf-life.

Louis Pasteur: a microbiologist of genius

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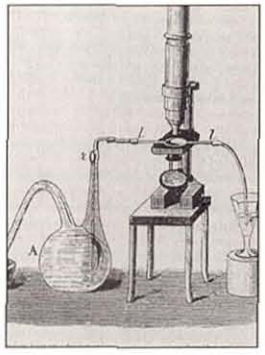

Microbiology of foods in modified-atmosphere packaging

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Introduction

The ability of modified-atmosphere packaging (MAP) to extend the shelf-life of foods has been recognised for many years. In the 1920s the Low Temperature Research Station showed that the shelf-life of apples could be increased by storing them in atmospheres containing low levels of oxygen and increased carbon dioxide, and in the 1930s beef carcasses were transported in atmospheres containing carbon dioxide, which approximately doubled the storage life previously obtained. It was Marks & Spencer in 1979 who paved the way for Britain's pre-eminence today in the world marketplace for modified-atmosphere products with its test launch of MAP meat.[1] Since then there has been a marked expansion in the use and market share of MAP, partly as a result of the increasing consumer demand for fresh and chilled convenience foods containing fewer preservatives. This has led to a significant increase in the range of products packaged in modified atmospheres. Today, foods packaged in modified atmospheres include raw and cooked meats, poultry and fish, vegetables and fruit, fresh pasta, cheese, bakery products, crisps, coffee and tea (**Figure 1**). The potential advantages and disadvantages of MAP have been tabulated by Farber in **Table 1**.[2]

There are several techniques by which the atmosphere surrounding a product can be modified and often the terminology is confusing. The four main techniques used today are as follows:

1. *Modified-atmosphere Packaging (MAP);* the replacement of air in a pack by a different mixture of gases, where the proportion of each component is fixed when the mixture is introduced, but no further control is exercised during storage.

2. *Controlled-atmosphere Packaging (CAP);* packaging in an atmosphere where the composition of gases is continuously controlled throughout storage. This technique is used primarily for the bulk storage of products and requires constant monitoring and control of the gas composition.

3. *Equilibrium-modified Atmosphere (EMA);* used primarily for fruit and vegetables; either the pack is flushed with the required gas mix or the produce is sealed within the pack with no modification to the atmosphere. Subsequent respiration of the produce and the gas permeability of the packaging allow an equilibrium-modified atmosphere to be reached.

4. *Vacuum Packaging (VP);* the product is placed in a pack of low oxygen permeability, air is evacuated and the package sealed. The gaseous atmosphere of the vacuum package is likely to change during storage (from metabolism of the product or micro-organisms) and therefore the atmosphere becomes modified indirectly.

Figure 1: A selection of MAP products.

Table 1: Potential advantages and disadvantages of MAP.[2]

ADVANTAGES

- Shelf-life increase of 50–400% possible.
- Economic losses reduced (longer shelf-life to spoilage)
- Products can be distributed over longer distances and with fewer deliveries, leading to decreased distribution costs.
- Provides a high quality product.
- Easier separation of slices.
- Improved product visibility.

DISADVANTAGES

- Visible added cost.
- Temperature control necessary.
- Different gas formulations for each product type.
- Special equipment and training required.
- Product safety to be established.
- Increased requirement for display space.

Modified from Farber[2]

The role of gases

The three major gases used commercially in MAP are oxygen, nitrogen and carbon dioxide, although several other gases have been investigated e.g. carbon monoxide, sulphur dioxide, nitrous oxide, ozone and chlorine. However, the use of these has been limited by safety concerns, legislation and negative effects on organoleptic properties and cost.

Oxygen (O_2) will generally stimulate the growth of aerobic bacteria and can inhibit

the growth of the strictly anaerobic bacteria, although there is a very wide variation in the sensitivity of anaerobes to oxygen.[2] Oxygen is very important in fresh meats to maintain myoglobin in its oxygenated form (oxymyoglobin), which gives fresh meat its bright red colour. Its presence may cause problems with oxidative rancidity or colour in some products (e.g. fatty fish and cured meats, respectively).

Nitrogen (N_2) is an inert tasteless gas with low solubility in both water and lipid. It is used to displace oxygen in packs so as to delay oxidative rancidity and inhibit the growth of aerobic micro-organisms.[2] Because of its low solubility it is used as a filler gas to prevent pack collapse which may occur in high CO_2-containing atmospheres.

Carbon dioxide (CO_2) is both water- and lipid-soluble and is mainly responsible for the bacteriostatic effect seen on micro-organisms in modified atmospheres.[2] The overall effect on micro-organisms is an extension of the lag phase of growth and a decrease in the growth rate during the logarithmic phase. This bacteriostatic effect is influenced by the concentration of CO_2, the age and load of the initial bacterial population, storage temperature and type of product to be packaged.[3] Although this bacteriostatic effect of CO_2 has been known for many years, the precise mechanism of its action is still not clearly understood. Farber[2] summarised the theories regarding the influence of CO_2 on the bacterial cell as:

1. Alteration of cell membrane function including effects on nutrient uptake and absorption.
2. Direct inhibition of enzymes or decreases in the rate of enzyme reactions.
3. Penetration of bacterial membranes, leading to intracellular pH changes.
4. Direct changes to the physicochemical properties of proteins.

With high-moisture/high-fat foods such as meat, poultry and seafood, excessive absorption of CO_2 can lead to the phenomenon known as 'pack collapse'. Increased in-pack drip is also caused by dissolution of the gas into the surface of fresh muscle foods which reduces their pH sufficiently to weaken the water-holding capacity of the proteins.[1]

Effect on microbial spoilage

In general, aerobic micro-organisms are sensitive to CO_2 and it is this, along with their requirement for O_2, that is utilised in MAP to control the spoilage of foods. Gram-negative bacteria are generally more sensitive to CO_2 than Gram-positive bacteria.[4] In chill-stored proteinaceous foods such as meat and fish, this generally results in the inhibition of the Gram-negative *Pseudomonas*, Enterobacteriaceae and *Acinetobacter/Moraxella*, whilst the Gram-positive lactic acid bacteria and *Brochothrix thermosphacta* become the dominant organisms. A comparison of the developing microflora on rainbow trout (*Oncorhynus mykiss*) stored aerobically or in MAP (80% CO_2/20% N_2) at 5°C is presented in **Figure 2**. Packaging in the 80% CO_2/20% N_2 inhibits growth of all the organisms examined, but particularly the Gram-negative Enterobacteriaceae and *Pseudomonas* sp.

As moulds have an absolute requirement for O_2, in foods where mould spoilage is the major concern, e.g. bakery products or hard cheese, packaging in an anaerobic MAP can be extremely successful in delaying spoilage. If CO_2 is used to produce the MAP there is also the additional benefit of the anti-bacterial and mould activity of CO_2.

Effect on microbial pathogens

Whilst the ability of MAP to extend the shelf-life of many products is well recognised, concern has been expressed by regulatory authorities, food industry groups and others that MAP may represent an undue safety hazard. The concerns are that suppression of the normal spoilage flora may result in an organoleptically acceptable product, whilst either allowing or enhancing the growth of pathogenic organisms. Historically, the non-proteolytic, psychrotrophic strains of *Clostridium botulinum* have been the major safety concern. These strains can grow and produce toxin without producing overt signs of spoilage, which may also be absent as a result of an inhibition of the normal spoilage flora. More recently, concerns have been expressed about the ability of the other psychrotrophic pathogens, e.g. *Aeromonas*, *Listeria* and *Yersinia*, to grow in MAP products.

Clostridium botulinum

Historically, MAP of fish and fish products has been the greatest cause for concern with respect to *Cl. botulinum*, and resulted in the US National Academy of Sciences recommending that, until the safety of the system was established, fish should not be packed in MAP.[5] This results from the isolation of all types of *Cl. botulinum* from marine environments, which, although highly variable by geographic location and season, is frequent enough that processors must assume its presence. Strains of type E and the non-proteolytic types B and F are the major concern in MAP as they are able to grow at temperatures as low as 3.3°C, albeit slowly, and, as they do not putrefy proteins, may not show obvious signs of spoilage. Numerous studies have examined the relationship between time to toxin production and signs of organoleptic spoilage for MAP fish, and these have been

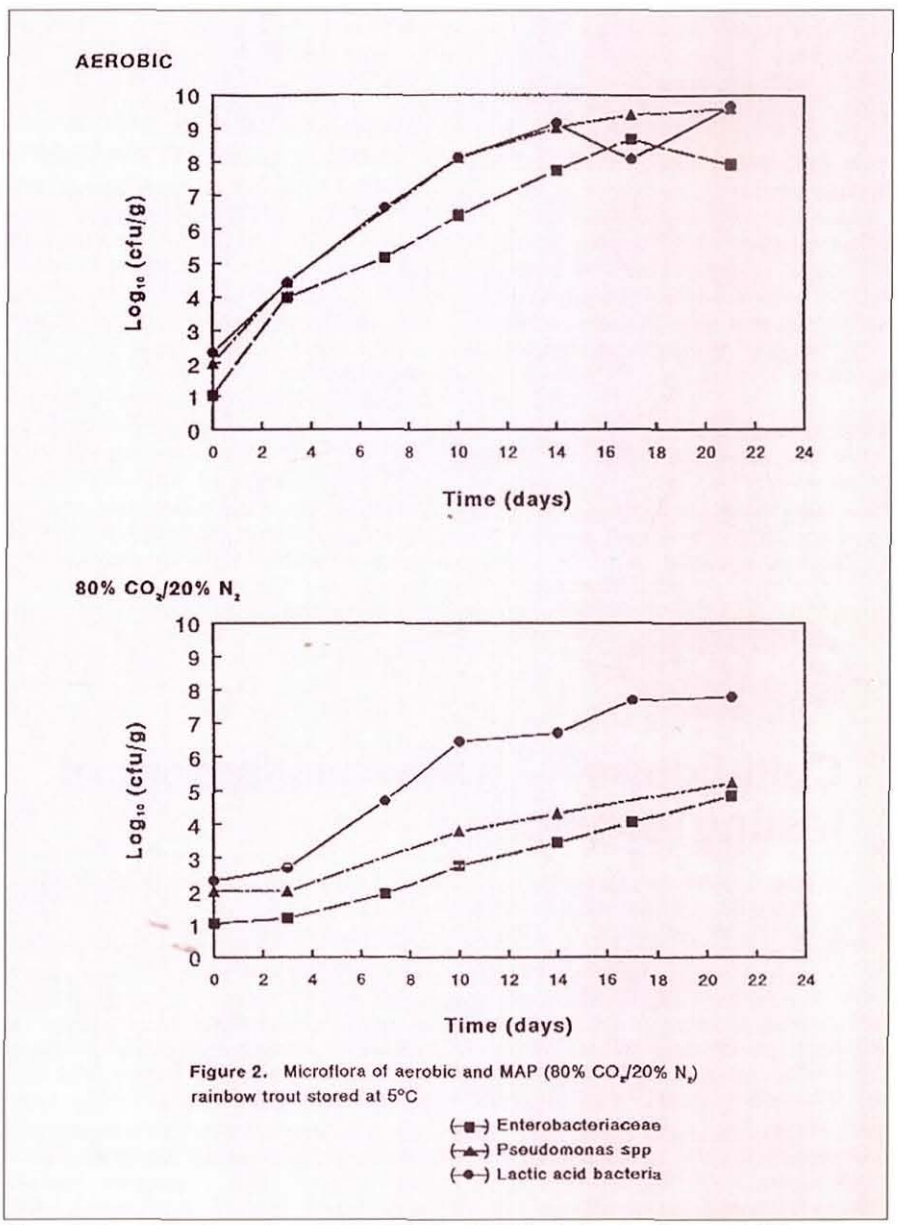

Figure 2. Microflora of aerobic and MAP (80% CO_2/20% N_2) rainbow trout stored at 5°C

Figure 2: Microflora of aerobic and MAP (80% CO_2/20% N_2) rainbow trout stored at 5°C.

reviewed.[3,6] Unfortunately, because of the many variables between studies, e.g. fish type, size and site of inoculum, temperature, season, atmosphere, etc., direct comparisons between the studies cannot be made. Stammen *et al*[6] in their review concluded that "*with few exceptions, at temperatures above 20°C, organoleptic spoilage coincided with toxin production in many fresh fishery products, regardless of the modified atmosphere used. However, at lower temperatures, organoleptic spoilage preceded toxin development in all fresh fish products except cod and whiting fillet held in either an air, vacuum or CO_2 atmosphere. This trend was seen at storage temperatures from 4–12°C. The time interval between toxin development and organoleptic spoilage of MAP fish products generally decreased as storage temperatures increased. In contrast toxin development preceded organoleptic spoilage in cod and whiting fillets packaged in 100% CO_2 and held at refrigeration temperatures. These products were still acceptable for consumption even though botulinal toxin was found in them.*"

Baker and Genigeorgis developed a predictive model from over 18,700 samples analysed over a 5-year period.[7] The utility of the model was demonstrated by its ability to predict the time before toxigenesis in inoculated fish stored under different MAs as reported in the international literature. Temperature explained 74.6% of experimental variation in the final multiple linear regression model ($3r^2$=0.883) but, surprisingly, the gaseous atmosphere was of little importance.

One approach that may provide the safety required for MAP of fish with respect to *Cl. botulinum* is the use of a pre-treatment in combination with MAP. Potassium sorbate, sodium chloride and irradiation in combination with MAP have all been shown to be effective.[6]

Other pathogens

Whilst there have been numerous studies on the effect of MAP on *Cl. botulinum* there have been few studies on the effect on the other foodborne pathogens, particularly the psychrotrophic strains.

At Leatherhead Food RA we have recently completed two EC projects that examined pathogen growth/survival on MAP fish and meat and their products. Studies with fish have concentrated on cod (*Gadhus morhua*) and rainbow trout (*Oncorhynus mykiss*) with storage at 0, 5 and 12°C. In no instance was the growth/survival of any of the pathogens examined greater than that in the aerobically stored control and frequently growth was reduced in MAP. Studies on meat have concentrated on raw beef but have also included cooked and dry-cured hams. The control atmosphere used throughout for meats was vacuum pack. As for fish, but with one exception, the growth/survival in MAP was never greater than that in the control and occasionally growth was reduced in MAP. The one exception was verotoxigenic *E. coli* on beef stored at 12°C, in which growth in one of the modified atmospheres examined was greater than that in the vacuum-pack control. Growth in the MAP was, however, reduced in comparison with that in aerobically stored product.

As found by others[8–10] we observed a CO_2-dependent bacteriostasis of *Salmonella* at chill temperatures. We did not, however, see the marked difference reported between aerobic and anaerobic MAPs on the growth of *Listeria monocytogenes*.[11] Our results with *Aeromonas* and *Yersinia* on beef differ from those reported for high-pH beef[12] and highlight the importance of assessing the safety of individual foods to be packaged in MAP.

Overall, our results and the majority of those reported in the literature indicate that the risks from the other foodborne pathogens in MAP are no greater and frequently are less than those from aerobically stored foods. These findings are substantiated by the excellent safety record, to date, of MAP.

The future

A number of recent developments offer the potential to improve further the safety of MAP and extend the technology available to a wider range of products. These developments include the use of pre-treatments in conjunction with MAP and the use of time-temperature indicators (TTI's), both of which may provide the assurance against the germination and outgrowth of *Cl. botulinum* that remains the major concern with respect to the safety of MAP. Mathematical modelling of microbial growth/survival in MAP foods and the development of specific HACCP plans will also afford extra assurance of the safety of MAP foods.

Possible negative factors that may affect the future of MAP are the environmental concerns about the use of packaging materials and the possible future requirement to label MAP foods.

Finally, the development of active or smart packaging—that is packaging films or agents included in the packs, which possess the ability either to absorb or to emit gases, vapours and odours[13]—offer the potential to extend the use of MAP.

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Louis Pasteur: a microbiologist of genius

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Introduction

This paper completes the trilogy of Koch–Lister–Pasteur and it is appropriate that the greatest of these three pioneers of medical microbiology is left to the last. The scientific achievements of Louis Pasteur (**Figure 1**) are simply too many for adequate comment within this limited space. **Table 1** summarises the more important events of Pasteur's life in chronological order, while the references will indicate where further details of his achievements can be found

What made Pasteur so successful in the many diverse fields of science he investigated? He certainly had a consuming ambition to succeed and he was not afraid to offend the conventional scientific opinions of his day. However, his apparent arrogance was based upon meticulous experimentation which gave his results great authority. Furthermore, he had an intuitive ability to get to the centre of complex problems. Once there, he was able to draw on his past experience so that a continuous line of progress can be traced throughout Pasteur's career.

Figure 1: Louis Pasteur as a young man with his wife Marie Laurent.

Table 1: Pasteur's life and major achievements.

1822	Born 27 December in Dole
1847	Doctorate degree in physics and chemistry
1848	First discoveries on crystal asymmetry
1849–1854	Professor of Chemistry, Strasbourg University
1854–57	Professor of Chemistry, Lille University
	First publication on fermentation (1857)
1857–1867	Director of Science, École Normale Supêriore, Paris
	Disproved spontaneous generation (1861)
	Discovered anaerobic bacteria (1861)
	Commenced work on silkworm disease (1865)
	Paper on wine diseases and pasteurisation (1866)
1867–1874	Professor of Chemistry, Sorbonne, Paris
	Elected Fellow of Royal Society, London (1869)
	Published book on silkworm diseases (1870)
	Elected member of Académie de Médicine (1873)
1876	Published book on diseases of beer
1877	First work on anthrax
1881	Published work on fowl cholera anthrax vaccination experiments
	first paper on rabies
1882	Elected to Académie Francaise
1885	First human vaccination against rabies
1888	Opening of the Pasteur Institute, Paris
1882	Seventy year jubilee at the Sorbonne
1895	Died 28 September, Garche, near Paris.

The chemist

Louis Pasteur was born in 1822 into a family of modest prosperity. Napoleon I had died the previous year in exile and the intense patriotism of Pasteur's father was clearly seen in the son. Pasteur's determination to succeed was revealed in his early academic achievements at the École *Normale Supériore, Paris, where he eventually* obtained his doctorate degree in physics and chemistry. His first research project brought him both fame and early recognition of his considerable talents. His investigation into the rotation of polarized light by crystals of tartic acid led to the discovery of asymmetry in the laevo- and dextro-rotatory forms of the crystals.

This discovery led in turn to a study of the relationship of optical activity to molecular and crystalline structure in stereoisomers. Pasteur was already looking ahead and formulating in his mind a universality of asymmetry in which the chemistry of dead and living matter could be differentiated by molecular asymmetry. Throughout his life Pasteur never completely lost his 'alchemist's dream' of solving the chemistry of life.

The industrial microbiologist

When later he took the chair of chemistry at Lille, Pasteur was asked to direct his teaching and research towards the industrial interests of the region. This agreed with his natural patriotism and guided his philosophy of science for the rest of his life. Pasteur saw no conflict between pure and applied science; it was simply a matter of applying one discipline to solve the problems of the other. Therefore, when asked to investigate the uncertain process of alcohol fermentation, he quickly developed an essentially practical experimental procedure. He detected optical activity in the sugar solutions and connected this with the presence of yeast cells and other minute bodies. This observation led to his conviction that fermentation was the activity of living things. He eventually proved this theory in the face of intense antagonism from contemporary chemists, such as Liebig, who thought fermentation was abiogenic chemistry. Pasteur went on to study lactic fermentation and devised the heating process, now called *pasteurisation*, that is used to prevent microbial spoilage.

Having established the effects of microbial activity in wine, milk and meat, Pasteur saw that his next task was to resolve the theory of the spontaneous generation of life. Although he claimed to have started with an open mind, his rigorous experimentation soon convinced him that microbial growth did not take place if microbes were properly excluded. The experimental detail and the conclusions drawn from them now rank as

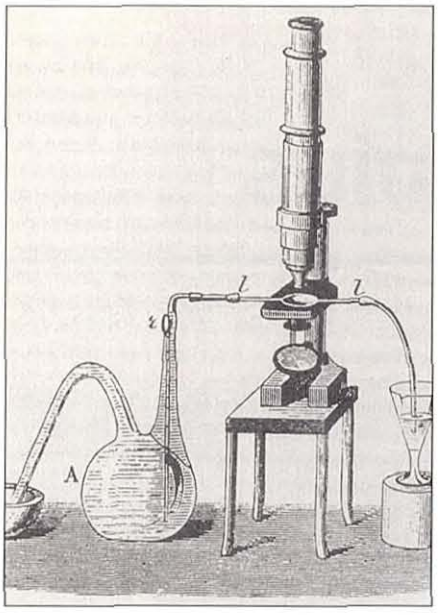

Figure 2: Apparatus for studying anaerobic bacteria.

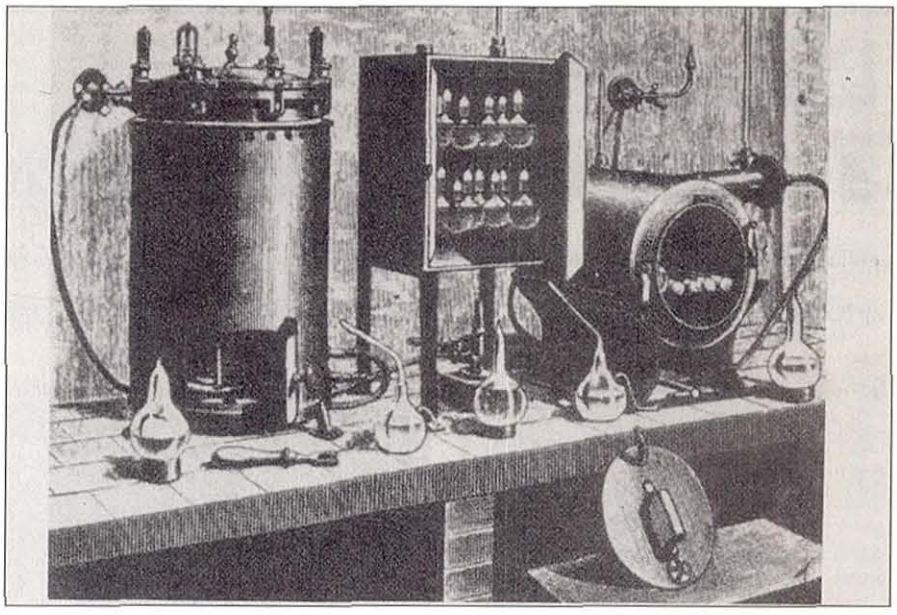

Figure 3: Autoclave, incubator and steamer as used in Pasteur's laboratory.

classical demonstrations of proof of sterility. The consequences of this work were applied directly to food and drink processing, then ultimately to contagion and disease.

Another example of his powers of observation and deduction occurred when he discovered anaerobic organisms. Whilst observing bacteria in sugar solutions under the microscope (**Figure 2**), he noted that those bacteria at the edge of the coverslip gradually lost their motility whilst those in the centre remained vigorously motile. This loss of activity was always associated with the production of butyric acid. Thus, he deduced that these butyric acid-producing organisms could not survive in the presence of oxygen. In 1861 the thought that life could exist in the absence of oxygen was quite revolutionary. However, Pasteur had previously observed the effects on alcohol production by yeasts growing in sugar solutions, both in the presence and absence of air. He used the term 'fermentation' to mean metabolism in the absence of air and 'oxidation' for metabolism in the presence of air. Oenologists will consider his later work on wine, commissioned by Napeleon III in 1865, to be the pinnacle of his genius. Pasteur showed that the oxidation of wine caused its instability, but a carefully controlled oxidation gave it a maturity. Step-by-step, Pasteur was treading a pathway which would eventually lead to the cause of human infectious diseases.

The pathologist

Starting with specific organisms and specific fermentations, he was soon to postulate that specific contagious diseases must have similar specific microbial causes. However, before he could start on this important phase of his life, another industrial crisis demanded his attention. A mysterious disease had attacked the French silkworm nurseries and by 1865 the silk industry was near to total ruin. Although knowing nothing about silkworms, Pasteur agreed to head the commission of investigation. During this investigation, which lasted for five years (1865–1870), Pasteur moved into experimental pathology and it was this work which would influence his future microbiological philosophy. Working in a small makeshift laboratory in Alès, he established that the silkworms had two separate diseases, *pebrine* caused by a parasitic protozoan and *flacherie*, a nutritional disease.

Having established the causes, he set about a vigorous teaching programme for the silkworm producers to raise hygiene standards and improve feeding procedures. The implications of this association between disease and malnutrition remained with Pasteur. He later returned to it when discussing human infectious diseases and the poor resistance of the malnourished.

However, this five-year period of intense activity and resounding success was not achieved without personal tragedy. During this period Pasteur suffered the death of two of his children, and also of his father, whom he regarded with great affection. In 1868 a severe cerebral haemorrhage left Pasteur with permanent paralysis of his left arm and leg. Such was his indomitable will-power that after a few weeks rest Pasteur returned to his primitive laboratory, high in the Cévennes mountains, to continue and ultimately conclude his work.

The medical microbiologist

It is possible to describe 1877 as a watershed in Pasteur's career (**Table 1**). From this

Figure 4: Louis Pasteur in later years examining a bacterial culture.

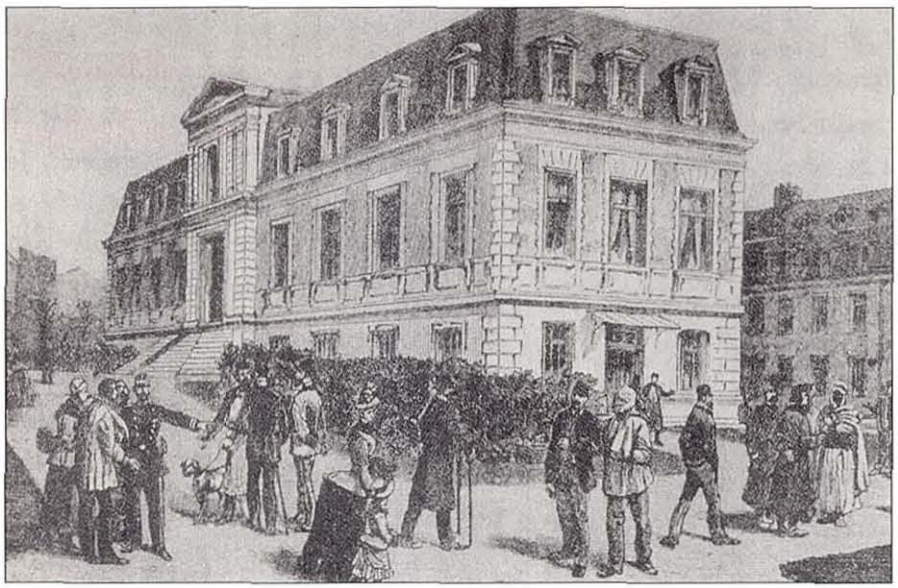

Figure 5: The Pasteur Institute in 1888.

time on, the whole of his attention would be devoted to human infectious diseases and, at 55 years of age, he was about to embark upon the most famous part of his outstanding career (**Figure 4**).

Pasteur had commenced his work on anthrax quite unaware of Robert Koch's investigations taking place in Germany. Using fluid cultures he was able to isolate the bacillus and demonstrate its virulence in laboratory animals. He then discovered the immunising effect of laboratory-attenuated cultures, which in turn led to the famous anthrax vaccination experiment at Pouilly le Fort in 1881. Pasteur was well aware of Jenner's work on smallpox, which had taken place a century earlier, and he was convinced that bacterial diseases could be prevented in the same way. Pasteur maintained the use of the word 'vaccination' for all his bacterial immunisation procedures.

Rabies

Following his anthrax experiments he studied the cause of fowl cholera (*Pasteurella multocida*) and demonstrated how this disease could also be controlled by 'vaccination'. However, it was the conquest of rabies that made Pasteur the outstanding scientist of his century. This ancient disease, first described by Hippocrates, was detested and feared throughout Europe at this time. Although Pasteur could not recognise the virus itself he was able to passage the agent through laboratory animals. By suspending infected rabbit spinal cords in sterile air, he was able to attenuate the virulence of the virus. His experimentation proved that these virus preparations could protect animals from fresh street virus inoculations. Pasteur was not happy about using animals for these tests and was extremely reluctant to extend his work into human cases.

However, in 1885 the decision was forced upon him when Joseph Meister, a young shepherd boy, was brought to Pasteur's laboratory. Joseph had recently been savagely bitten by a rabid dog and there was little hope that he would survive beyond the few months incubation time for rabies. Reluctantly, Pasteur commenced a series of injections using spinal cords which contained the virus in greater degrees of virulence. Joseph Meister's life was saved and he survived until 1940, when he died protecting Pasteur's crypt during the occupation of Paris.

This success was soon to be repeated when a second victim, a young man named Jean Baptiste Jupille, was bitten whilst heroically protecting children from a rabid dog. Jean Baptiste was similarly treated, and the public's imagination was fired by the combination of the young man's heroic deed and his miraculous recovery. By 1886, only one year after his first success, over 2,000 people had been successfully treated by this new method. In 1888 the Pasteur Institute (**Figure 5**) was opened as a centre for the treatment of contagious diseases and was a fitting tribute to Pasteur, then 66 years of age, and his outstanding achievements.

Pasteur suffered increasing ill-health during his final years and he died in 1895 at the Chateau Villeneuve-l'Etang, Garche, near Paris. His bedchamber is still preserved, unaltered from the time of his death. The room itself is starkly simple and the bed is surprisingly small.

Epitaph

Much of Pasteur's work from his 47 years of research remains current today. His crystal stereo-isomer investigations are now being further developed in the pharmaceutical industry. Monochiral forms of drugs have been shown to be superior to racemic forms, since they are free from the side-effects produced by the opposite chiral structure.

Pasteur stated that science was the dominating passion of his life, and described himself as an enthusiast. He considered the work *enthusiasm*, from the Greek meaning 'a God within' to be one of the most beautiful words in the language. He said 'The grandeur of the acts of men is measured by the inspiration from which they spring. Happy is he who bears a God within'.

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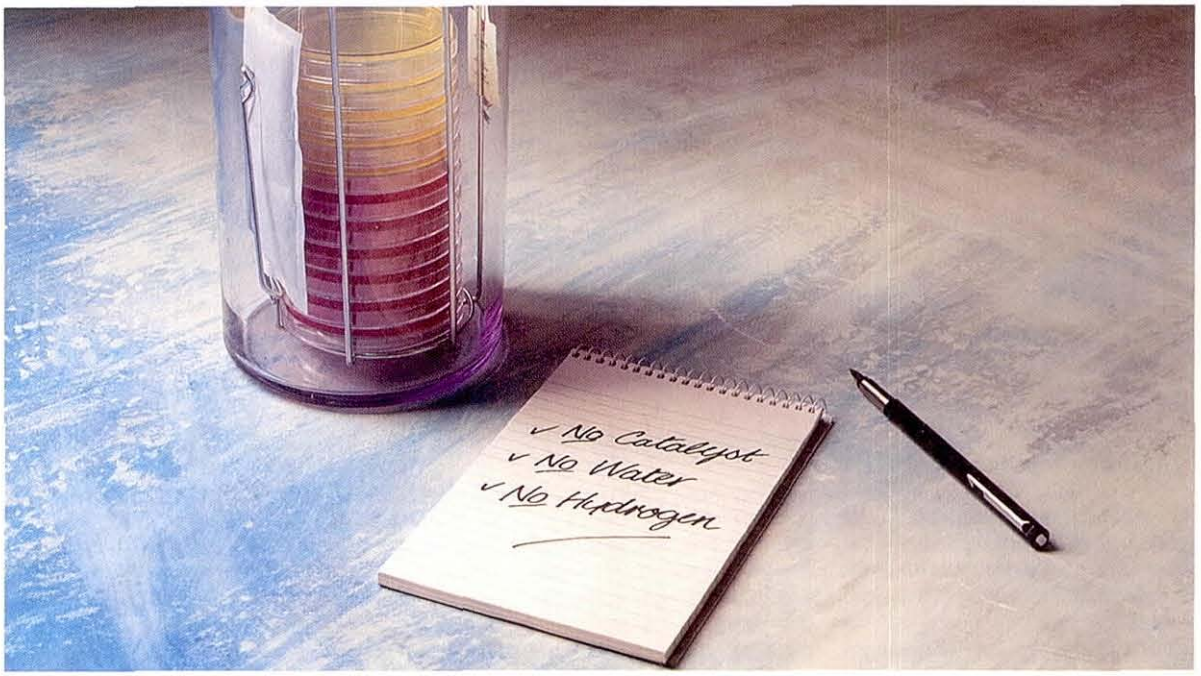

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