6
Safety in Microbiology: a Review

C. H. COLLINS

Public Health Laboratory, Dulwich Hospital, East Dulwich Grove, London SE22 8QF, UK

Introduction

Several generations of scientists have been aware of the health risks involved in work with certain microbes. Attention was first drawn to the problems and hazards of laboratory-associated infections by German workers long before the Second World War (Paneth, 1915; Kisskalt, 1929). Stories of microbiological martyrdom, ranging from the emotive (de Kruif, 1926, 1933) to the sober (Hunter, 1936) aroused more public interest, however, than the factual accounts of laboratory-acquired infections, over 400 of which are cited by Collins (1983). The number of individuals affected is difficult to determine because many cases were probably missed and others, for various reasons, were not reported. However, a world-wide survey started in 1950 by Sulkin and Pike (1951) and continued until 1978 (Pike, 1978, 1979), revealed by literature research and questionnaires that there had been (at least) 4079 infections with 168 deaths. These numbers included not only single cases, but institutional or common source outbreaks each involving more than 20 people.

Since these figures were published they have been augmented by further accounts, e.g. of Banerjee, Gupta and Goverdhan (1979) who reported 87 cases of Kyasanur Forest disease (caused by KF virus), all in, or associated with, one laboratory; and of over 30 cases of laboratory-acquired typhoid fever in different parts of the United States, and all connected with the distribution of proficiency-testing material (Anonymous, 1979). There have also been many more single or single-figure incidents.

Surveys in the United Kingdom have been concerned more with morbidity among laboratory workers than with the identification of laboratory-acquired infections. This is understandable in view of the difficulties in identifying these infections. Not only is there the possibility of under-reporting, mentioned above, but there are the problems of connecting an infection, especially one with a long or uncertain incubation period, with an incident which has been forgotten or never recorded. These problems have been considered by Phillips (1961) and Collins (1983). Thus Reid (1957) investigated 153 cases of tuberculosis among laboratory workers and concluded that the various categories of such workers

Biotechnology and Genetic Engineering Reviews—Vol. 1, February 1984
0264-8725/84/01/141-25$10.00 + $0.00

were from two to nine times more likely to contract that disease than matched controls. In a single year (1971) in England and Wales, Harrington and Shannon (1976) found 21 cases of tuberculosis, 38 of hepatitis and 45 of shigellosis among about 22000 laboratory workers who replied to a questionnaire. In a similar survey for the year 1979, Grist (1981) identified five cases of tuberculosis, three of salmonellosis, one each of malaria, shigellosis and hepatitis and four of chicken pox. For 1980–81 (Grist, 1983) he recorded nine cases of hepatitis, 19 of tuberculosis, 13 bowel infections, and seven 'others'.

Between the surveys of Harrington and Shannon and those of Grist there were two important events that stimulated interest, concern and positive action in the United Kingdom. The first of these was an increased incidence of hepatitis B in medical laboratory workers: 17 cases in 1970–72 (Grist, 1975). The second was the laboratory origin of smallpox cases in London in 1973. While the first of these was largely a local problem, the smallpox incident had world-wide repercussions, especially when it was followed, in 1978, by another incident in which a Birmingham laboratory was involved.

At about the same time, and on an international scale, public concern was aroused about two other potential microbiological hazards. There were fears that some of the 'new' diseases, like Lassa and other haemorrhagic fevers, which have a high mortality rate and for which there was no vaccine nor specific treatment, might be spread rapidly by air travellers. The other, which appeared to frighten some scientists as much as the less well-informed public, was the possibility that the newly developed recombinant DNA research might produce 'doom bugs' which would threaten the whole human race. Although both of these fears are now known to be largely conjectural, they contributed to the pressures which compelled governments and other bodies to act, even if only to set up committees.

Events in the United Kingdom

We are concerned here with official actions that were designed to regulate the activities of microbiologists, not only those who work with viruses and bacteria known to be highly pathogenic, but also those who use comparatively harmless organisms or microbes which have never been known to cause human disease. It is an unfortunate fact of life that restrictions which might reasonably be placed at one end of the scale are frequently applied to the other, because the officials who enforce them are unfamiliar with the principles involved and do not trust the scientists to regulate their own activities. Nor is this lack of trust entirely unmerited. Although most scientists are indeed responsible citizens, most of us who have been engaged in the study of microbiological hazards have encountered the biochemist with no microbiological training, who engages, or proposes to engage, in 'bucket bacteriology' with pathogenic micro-organisms and no precautions. Two incidents come to my mind: we were asked to recommend a medium for growing *Brucella melitensis* in winchester bottles in a college incubator so that the organisms could then be separated in an open continuous flow centrifuge; and to advise on the proposal to work with *Salmonella typhi* in a room with sole access through a staff canteen.

Although smallpox is officially extinct, and all known world stocks of virus have been deposited in specified laboratories, a senior WHO scientist expressed to me his fears that 'there may still be an ampoule of unlabelled virus left in the bottom of a deep freeze by some guy who has forgotten all about it; someone else finds it and then what?'.

The smallpox incident in London in March 1973, which claimed the lives of two people who had no connection with the smallpox laboratory, was the subject of a detailed and expensive inquiry (Report, 1974). In November 1973, the Secretary of State for Social Services set up a Working Party on the Laboratory Use of Dangerous Pathogens under the chairmanship of Sir George Godber, then Chief Medical Officer. This Working Party reported 18 months later (Report, 1975a) and made certain recommendations. It also appended a Code of Practice for laboratories working with the most dangerous organisms, designated Category A pathogens. One result was the formation of the Dangerous Pathogens Advisory Group (DPAG) which then published its constitution and a revised Code of Practice (Department of Health and Social Security (DHSS), 1976). The activities of this group were confined to advising on the suitability of particular laboratories for work with viruses such as those of smallpox and haemorrhagic fevers, and on the precautions that should be taken.

The (Godber) Working Party also recommended that a Code of Practice should be established for the less dangerous pathogens, designated Category B, which are encountered mainly in clinical and diagnostic laboratories. In 1975 a Working Party to formulate a Code of Practice for the Prevention of Infection in Clinical Laboratories was convened with Sir James Howie, formerly Director of the Public Health Laboratory Service, as Chairman. This Working Party presented a Report, which was never published, making several recommendations, none of which has been effectively taken up, and a Code of Practice (DHSS, 1978) which was circulated to all laboratories and to individual laboratory workers in the National Health Service. The Code of Practice, now known as the Howie Code, was well received among laboratory workers (*sensu stricto*) in clinical laboratories but many objections to its provisions and requirements were made by administrators and others who were not exposed to laboratory infections.

The (Howie) Working Party recognized the temporary nature of parts of their Code and that progress and new developments would soon make some of its provisions out of date and in need of revision. They recommended the setting up of a 'small permanent advisory group to keep this Code of Practice up-to-date, to ensure that it remains relevant to the needs of safety in clinical laboratories ... and to give advice and such other help that may be requested'. Unfortunately, this very sensible recommendation was not accepted. There have been attempts to tinker with the Howie Code but the printed words remain, regrettably, unchanged.

The Health Departments in the UK laid down a time scale for National Health Service laboratories to meet the requirements of the Howie Code. Coincidentally it became possible and practicable for the Health and Safety Executive (HSE) to apply the Health and Safety at Work Act to clinical and

other laboratories. The Howie Code was a convenient, although not mandatory, instrument for the Inspectorate to use. Thus the Inspectors were not only applying the Act, they were fulfilling the Health Departments' ambitions for them. These moves aroused some controversy and two non-governmental bodies were formed to consider the effects of the Code and its application.

The first of these was the Joint Working Party on the Prevention of Infection in Clinical Laboratories. Its members are drawn from societies and organizations concerned with clinical pathology, i.e. who are directly affected by the Howie Code. This committee is uniquely placed for advising the Health Departments, the Health and Safety Executive and clinical laboratory workers in general, but so far it has not published any alternative code of practice.

The other group, formerly known as the Joint Coordinating Committee for the Implementation of Safe Practices in Microbiology, is now known as the Microbiological Consultative Committee (MCC). Its members represent the learned societies that are engaged in non-clinical microbiology, for which the Howie Code was not designed and cannot be sensibly applied. This committee provides a channel of communication between members of the societies and official bodies such as the Health and Safety Commission, the Department of Health and Social Security, the Genetic Manipulation Advisory Group (GMAG: *see below*) and the Research Councils. Its aim is 'to promote the highest standard of cost-effective safe practice with the minimum of bumbledom'. It maintains close links with the Joint Working Party on the Prevention of Infection in Clinical Laboratories and with the Royal Society safety groups. A major task of the MCC has been to offer practical advice to local safety committees who were charged under the Safety Representatives and Safety Committee Regulations (Statutory Instrument 1977, No. 500) with the responsibility of supervising safety procedures in many establishments which had previously been exempt from statutory regulations. Although each local safety committee is expected to formulate its own code of safe practice, the MCC published its guidelines for microbiological safety in 1980, and because of its favourable reception, not only among laboratory workers, but also by HSE, GMAG and the Association of Scientific, Technical and Managerial Staffs, a second, revised edition was produced two years later (Microbiological Consultative Committee, 1980, 1982).

In 1978 there was another death from smallpox associated with a laboratory in Birmingham, the Director of which was a member of DPAG. The subsequent inquiry (Report, 1980) called into question the duties of that group and, as the result of recommendations made by a committee of permanent officials of DHSS and HSE, a new and larger body was created. This is the Advisory Committee on Dangerous Pathogens (ACDP) whose remit covers not only the truly dangerous pathogens, formerly the province of DPAG, and the less dangerous pathogens considered in the Howie Code, but in spite of its name, organisms which are not pathogenic at all! The ACDP has already published and invited comments on its first report (Report, 1983). Apart from venturing into the minefield of classifying micro-organisms into groups on the basis of hazard (considered later in this review), it would seem that the ACDP proposes to construct a new code of practice which will embrace all fields of microbiology.

Personally, I do not think that it is possible for any one group of individuals (unless it is a very large group indeed, which creates its own problems) to formulate a code which will satisfy the needs of workers in such diverse laboratory activities as work with very dangerous viruses, clinical microbiology, control of microbial spoilage in food, and the industrial use of organisms of any kind. It would be more realistic to have existing codes such as those mentioned above brought up to date by very small groups of individuals whose experience in those particular fields cannot be challenged. This would have the added advantage of avoiding vested and political interests. The codes might then be linked in one publication, suitably cross-referenced and containing an abstract of common ground.

Events outside the UK

The real or conjectural hazards of the rapid movement, e.g. by air transport, of dangerous pathogens and infected individuals, as well as recombinant DNA material, across national boundaries and into areas where they might cause epidemics, was the concern of some consultations held by the World Health Organization (WHO) in Geneva in 1976. The possibility of aircraft accidents, releasing pathogens of medical or economic importance into the environment, was also considered. The outcome of these discussions was the creation by WHO of the Special Programme on Safety Measures in Microbiology. Small international groups of advisors and consultants with experience in specific hazards in microbiology were convened. One group has produced guidelines for the management of accidents involving micro-organisms (WHO, 1979a, 1980) and another, minimum standards for laboratory safety in microbiology (WHO, 1979b). Other material relating to microbiological safety problems has been circulated but not yet formally published and a *Laboratory Biosafety Manual* has been printed (WHO, 1983).

Although activities in the field of microbiological safety have been patchy in Europe there have been strenuous efforts particularly in relation to biotechnology laboratories in the Federal Republic of Germany (FDR), in the Netherlands, and by the European Federation of Biotechnology, which, of course, includes the United Kingdom. The committee on Microbiological Safety of the Dutch Society for Microbiology has circulated a set of laboratory safety guidelines. Laboratory safety in the FDR is covered by an impressive but confusing set of regulations. Nevertheless, the Committee on Biotechnology of the Deutsche Gesellschaft für Chemisches Apparatwesen (DECHEMA) has produced a study document on safe biotechnology. Neither of these has yet been published formally but are regarded as very useful documents by the Safety in Biotechnology Group of the European Federation of Biotechnology. Members of this group are using the DECHEMA document as the basis for a set of guidelines.

American microbiologists, who have had their share of laboratory accidents and laboratory-acquired infections, have not had the trauma of committee decisions. They have worked within the framework of rules and recommendations made by professional microbiologists employed by the Centers for Disease

Control and the National Institutes of Health (United States Public Health Service (USPHS), 1974a, b, c).

Action on genetic manipulation experiments

The technique of transferring genetic material from one organism to another and allowing the latter to reproduce so that it will 'express' characteristics of the former is variously known as genetic manipulation, genetic engineering, bioengineering, gene engineering and recombinant DNA research. Fears that these experiments could produce new and highly pathogenic micro-organisms that might escape into the community were voiced by a group of scientists (Berg *et al.*, 1974) who, in a letter to *Science*, called for a moratorium on all such activities. The Berg letter had national and international consequences.

In America an Azilomar Conference considered that certain experiments should not be done (*see* Berg *et al.*, 1975) but that other work could continue with certain specified precautions. The National Institutes of Health Recombinant DNA Advisory Committee produced its guidelines a year later (USPHS, 1976, 1980a) and then a supplement, which included a great deal of additional information on microbiological safety in 1978 (USPHS, 1978). Thereafter, pending decisions by Congress about legislation on genetic engineering, the whole problem seemed to become a political football.

In the UK a Working Party on the Experimental Manipulation of Micro-organisms with Lord Ashby as Chairman concluded (Report, 1975b) that research should be continued, but with rigorous safeguards. A Working Party on the Practice of Genetic Manipulation, Chairman Sir Robert Williams, was then convened. It described categories of risk, produced a code of practice, with conditions for containment and recommended the formation of a Genetic Manipulations Advisory Committee (GMAG) to which intending workers in this field would be required to submit their experimental protocols (Report, 1976). GMAG was indeed constituted, in 1976, and has since then visited and advised scientists on genetic manipulations. In 1978 effective control passed to the Health and Safety Executive (HSE) under the Health and Safety (Genetic Manipulation) Regulations and scientists were required to give notice of their intentions and activities to HSE as well as to GMAG.

GMAG published a new categorization scheme in 1978, based on risk assessment, and a new Code of Practice in January 1981 (slightly amended later the same year). At present the future of GMAG is under review because, among other considerations, time and experiments have shown that the hazards of this kind of work, always conjectural, are now regarded as overstated.

In addition, there is now more emphasis on large-scale industrial applications of the research. HSE inspectors, who have experience of production methods in many fields, are, perhaps, better qualified than academic scientists to monitor the industries.

Activities in other countries have followed on much the same lines, with varying amounts of political concern. The Economic and Social Committee of the European Economic Community considered the subject in 1981 without arriving at any particularly new conclusions.

The Assessment of Risk

> 'If reasonable precautions are to be taken against laboratory-acquired infections it is necessary to assess realistically the hazards that might be imposed on the laboratory worker and the community during and as a result of work with any particular micro-organisms. It is a waste of time and resources to take elaborate precautions when the risks are negligible, but foolish to take none if they are considerable. The precautions should be appropriate to the organisms being investigated and the techniques used.' (Collins, 1983).

To assess the risks in order to determine the precautions, the following information is needed:

1. The history of the micro-organisms in so far as their ability to infect laboratory workers is concerned;
2. The routes of infection or the ways in which micro-organisms can enter the body of the laboratory worker (as distinct from the ways in which infection is spread in the community);
3. The dose required to initiate infection;
4. The availability of vaccines for preventing or attenuating infections and of specific therapy in case infections do occur;
5. The ways in which micro-organisms can 'escape' from their containers.

This information will enable:

1. A hierarchial classification of micro-organisms to be made according to the risks they offer to the laboratory worker, and, through him and his work, to the community;
2. Suitable barriers to be placed around the workers to protect them and around the organisms to 'contain' them. The nature and extent of these barriers would be determined by the organisms handled and the nature of the work.

In all this, one must not lose sight of host susceptibility, which varies with age, health and medication, and it must be assumed that the potential hosts, i.e. the laboratory workers, are healthy adults of working age, who are not receiving steroids or immunosuppressive drugs.

HISTORY OF LABORATORY-ACQUIRED INFECTIONS

It has long been appreciated that some micro-organisms (the term here includes viruses) are much more likely than others to infect those who work with them. *Table 1* shows the 'top ten' in Pike's (1978) survey, with *Brucella* in the lead. At the other end of the scale, in the same period *Vibrio parahaemolyticus* was incriminated only once. Nevertheless, it has become fashionable, among non-microbiologists and financial overlords, to discount as anecdotal the surveys and accounts of laboratory-acquired infection by Sulkin and Pike (1951), Sulkin (1961), Pike, Sulkin and Schulze (1965); Pike (1976, 1978, 1979) as well as the more restricted reports of Reid (1957), Harrington and Shannon (1976) and the

Table 1. The 'top ten' laboratory acquired infections.

Infection	*No. of cases*
Brucellosis	426
Q fever	280
Hepatitis	268
Typhoid fever	258
Tularaemia	225
Tuberculosis	194
Dermatomycosis	162
Venezuelan equine encephalitis	146
Psittacosis	116
Coccidioidomycosis	93
TOTAL	2168

Reproduced by permission of the author and publishers from Pike, R. M. (1978). *Archives of Pathology and Laboratory Medicine* **102**, (July), 333–336; Copyright 1978, American Medical Association.

series by Grist (1975–83). Indeed, Grist's figures, which show a dramatic decline in the incidence of hepatitis B among laboratory workers (especially biochemists and haematologists, who are most at risk), have been used as 'evidence' that all other laboratory-acquired infections have declined to the point that microbiological safety, as required by the Howie Code, is not cost effective (Cohen, 1982). One must agree, up to a point, with Williams (1981) that safety has become a bit of a 'bonanza', but it is very unwise to ignore these earlier accounts (summarized by Collins (1983)), although they have no factor in common and have not been subjected to statistical examination, unlike those of Harrington and Shannon and of Grist. There can be no argument whatever for not accepting the evidence of the more recent surveys.

ROUTES OF INFECTION

The ways in which micro-organisms can enter the human body and perhaps initiate infections are well known to practitioners in community medicine. In the laboratory, however, the route may be different. An example of this is brucellosis. Among the general public (veterinarians are an exception) this disease is usually acquired by drinking unpasteurized milk from infected animals. In the laboratory it is almost invariably acquired by the inhalation of the organisms released in aerosols during certain manipulations.

The eye is seldom a portal of entry outside the laboratory but there is adequate evidence and an impressive list of infections acquired in this way by laboratory workers (Papp, 1959; Collins, 1983).

The percutaneous route also is almost peculiar to laboratory workers. Accidents in which individuals stab or prick themselves with infected hypodermic needles or broken, contaminated glassware are not uncommon (Phillips, 1969; Pike, 1976). Apart from overt accidents, organisms may enter through cuts and scratches or microscopic abrasions in the skin as a result of contact with infected droplets or splashes, which might well pass unnoticed.

Infection by the oral route is almost invariably associated with aspiration during mouth pipetting and recorded incidents go back to the beginning of this century (Pike, 1978; Collins, 1983). On the other hand, although eating, drinking

and smoking in laboratories are rightly regarded as unhygienic and undesirable activities, there is little but anecdotal evidence of infections acquired in this way in the laboratory (Lubarsch, 1931; Pike, 1979; Collins, 1983).

INFECTIOUS DOSES

Although little is known about the numbers of organisms required to initiate naturally acquired infection, some information, derived from experiments on volunteers, has been accumulated at the National Institutes of Health (USPHS, 1978). The results show, for example, that 10 particles of *Coxiella burnetii* or *Francisella tularensis* are sufficient to initiate infection, but that more than 1300 anthrax organisms are necessary. At least 32 particles of adenovirus 24 caused infection by the ocular route. By the percutaneous route (subcutaneous or intradermal) one particle of Venezuelan equine encephalitis virus, three of *Rickettsia tsutsugamushi* and 57 of *Treponema pallidum* were required. Infection by the oral route required 10^5 organisms for typhoid fever; 10^8 for cholera and 10^9 for shigellosis.

The smaller of these doses demonstrates the extreme vulnerability of laboratory workers who handle these organisms. The larger doses are easily achieved in small droplets of culture material. In this context it should be noted that pathogens such as *Salmonella* serotypes, certain staphylococci and streptococci are frequently present in sub-infective doses in foodstuffs, animal by-products etc. and are easily concentrated into infectious doses by the ordinary cultural methods employed in quality control, and that their colonies may not be recognized.

PROPHYLAXIS AND THERAPY

Although many vaccines are available and are of undoubted value in the prevention of naturally acquired infection, it is by no means certain that they will prevent infection in laboratory workers who may be exposed to very large doses of micro-organisms, or if those organisms enter the body through an unusual route. Nor does vaccination automatically confer immunity. Even when vaccines exist, it may not be possible to give them to some individuals, e.g. those who are sensitive to egg products. Nor is it possible or practicable to vaccinate all laboratory workers against all the organisms they are likely to encounter. There remains, furthermore, a whole group of viruses for which no vaccine is available, and even some bacterial vaccines are known to be relatively ineffective.

In spite of remarkable advances in antibiotics and chemotherapy, and the high hopes expressed for interferon compounds, there are still a number of diseases to which laboratory workers are exposed where no useful therapeutic measures are available.

'ESCAPE' OF MICRO-ORGANISMS

Accidents in which organisms were injected or ingested, spilled or dropped on

to the skin, and the bites and scratches sustained from arthropods and mammals during laboratory manipulations, are well recorded, but account for only about 20% of the total number of infections (3921) in Pike's survey (Pike, 1976); Pike considered that the causes of the other 80% were a matter for speculation. However, between 1947 and 1962, a period which Kubica, in his lectures on biosafety at the Centers for Disease Control refers to as 'the frightening fifteen years', an impressive number of papers appeared which described how micro-organisms, in aerosol clouds or in dried material, are dispersed into the laboratory air during many ordinary laboratory manipulations. These technique- and equipment-related hazards include work with bacteriological loops, pipettes, centrifuges, homogenizers and hypodermic syringes, harvesting of eggs, and opening culture dishes, tubes and screw-capped bottles. These investigations did not stop at Kubica's 1962 deadline but continued for the next two decades. It is now clearly understood that the smaller of these aerosol droplets dry rapidly, leaving their nuclei of bacteria or virus particles freely floating in the air, to be moved around rooms and buildings on quite small air currents. If such nuclei are less than 0·5 μm in diameter and are inhaled, they pass into the lungs and may initiate an infection. Larger droplets and particles sediment rapidly and contaminate hands and work surfaces, from which they may be transferred to the eyes or the mouth.

Classification of micro-organisms on the basis of hazard

Armed with all this information it should be possible to place micro-organisms in groups according to the risks they offer to those who work with them and to the community if they 'escape' from the laboratory. If we accept four possible groups we can define them as follows:

1. Those that are not known to cause human disease or which do so only under special or unusual circumstances.
2. Commonplace pathogens that are usually present in the community but which may be handled quite safely by competent workers using good techniques and provided with adequate equipment. Prophylaxis and specific therapy are available.
3. Pathogens that can cause serious disease in laboratory workers, are comparatively rare in the community but offer little threat to it if they escape. These include organisms most likely to cause infection by the air-borne route (inhalation) as well as those capable of initiating infection by very small numbers and any route. Prophylaxis and specific therapy are available.
4. Pathogens that can cause very serious, often fatal disease in laboratory workers and which might give rise to epidemics. Neither vaccines nor specific therapy are available.

Several reasonably successful attempts have been made to classify micro-organisms on this basis and three of these are shown in *Table 2*.

The US system of classification evolved gradually between 1969 and 1974 and recognized four classes, 1—4 in ascending order of risk. In the UK

Table 2. Summary of systems for classifying micro-organisms on the basis of hazards to laboratory workers and the community, and the type of laboratory required.

	Low ⟶ *Hazard* ⟶ *High*			
USPHS (1974)	Class 1 none or minimal	Class 2 ordinary potential	Class 3 special, to individual	Class 4 high, to individual and community
UK (DHSS 1978)		Category C no special potential	Category B special, to individual	Category A high, to individual and community
WHO (1979)	Risk Group I low individual low community	Risk Group II moderate individual, limited community	Risk Group III high individual low community	Risk Group IV high, to individual and community
Appropriate laboratory	Basic	Basic	Containment	Maximum containment

From Collins (1983) by courtesy of Butterworths Ltd.

classification there are three categories, A, B, C in descending order of risk. Category A, the 'dangerous pathogens' were defined by the Godber Working Party (Report, 1975a) and the DPAG (DHSS, 1976). The less hazardous pathogens, in Categories B and C, were defined by the Howie Working Party (DHSS, 1978). There is no fourth category in this classification because the terms of reference of the Howie Working Party restricted it to 'infections' and a 'Category D' would have contained non-pathogens.

Although I was involved in formulating the UK system, I now consider that of the WHO (1979a, b) to be the best. Like the US classification, this has four classes—Risk Groups I–IV in ascending order of risk. It is the simplest system and has a wider and more international application than the others. Individual states, however, are still free to go their own way. The Dutch have their own scheme and two new US schemes have been proposed since that of 1974 (USPHS, 1981, 1983). Both are complicated and neither has found much favour among American microbiologists, but one seems to have been adopted at least in part by the ACDP (Report, 1983).

LISTS OF MICRO-ORGANISMS

Having participated in the construction of three sets of lists, I repeat my observation (above) that this area is a 'minefield'. It is not possible to create universal lists because of the uneven geographical distribution of the pathogens themselves, their vectors and reservoirs, and of variable standards of hygiene. An organism which merits a place in a high risk group in one country may deserve only a low category in another. It was for these reasons that the WHO workers felt unable to present lists, and recommended that each member state should create its own. Various other problems arise, however, in compiling 'local' lists.

There is a tendency, on the part of some committees that make lists, to elevate

into higher risk groups organisms of which they have no personal experience and which have been used industrially without incident for decades. In addition, organisms are sometimes allocated to risk groups or categories at the generic level. This is too broad. One should not argue, for example, that because one organism is a pathogen, all other species in the same genus should be in the same risk group. List-makers should also guard against too many 'exceptions' or these may well outnumber the species or strains in the main list, thus bringing it into disrepute.

It should also be possible for an organism to be listed in two different risk groups, depending on the amount used and the technique employed. Such differences occur between, for example, diagnostic and quality control laboratories on the one hand, and industrial research establishments on the other. Such a system is used in the newer American classifications (USPHS, 1981, 1983) but it makes them unwieldy and complicated. It would be better, as suggested above, to have separate codes for the various applications of microbiological work, each with its own but strictly relevant lists. In addition, at the diagnostic and 'pure' research levels in parasitology it should be recognized that certain stages in a life cycle may be more hazardous than others to the laboratory worker and this should be reflected in the categorization.

There are three possible ways of listing organisms within the risk groups or categories. One is to name, species by species and strain by strain, those that are known to be pathogenic and to allocate them to the three (or two) highest groups; and then to say that all others are in the lowest group. This approach was used in the US (USPHS, 1974, 1980b) and the UK (Report, 1975a; DHSS, 1978). An alternative would be to attempt to name all the organisms in the lowest-risk group as well. Although this idea has been put forward, hopefully, as a desirable long-term solution, it would be a monumental task and would require several standing committees. It appeals only as an exercise in bureaucracy.

The third way would permit lists of organisms in the lowest group but only in a well-defined context, e.g. in a particular field or industry. Review and revision of the lists would then be a comparatively simple task, easily done by the small groups of experts referred to above who would keep the appropriate code of practice up to date. Such a list, within a defined context, is presented in *Table 3*. This contains only the organisms discussed in a particular book (Collins and Lyne, 1984). Within such a context it is possible to use the expression 'other species'.

Problems also arise with 'difficult' and 'new' organisms. An example of the first is the hepatitis B virus, which is 'difficult' only because of the high incidence of laboratory- and hospital-acquired infection in 1969–71, which created so much emotion that the Howie Working Party were compelled by the threat of a minority report to place this virus (and material that might contain it) in a higher category than it deserved, thus getting out of step with other countries. Further attempts to place this virus in its appropriate category have failed, because no committee has yet grasped the nettle and the subject has become a political chess-piece. Members of the 'high-category' lobby still demand the kind of protection known to be ineffective (microbiological safety cabinets;

Table 3. Risk Groups of some genera and species discussed in one particular book (Collins and Lyne, 1984). Where only the generic name is given all the species mentioned in the text may be treated as if they belong to the Risk Group specified.
(Courtesy of Butterworths Ltd).

Bacteria

Organism	Risk Group	Organism	Risk Group
Acetobacter	I	*Gemella*	I
Achromobacter	I	*Gluconobacter*	I
Acinetobacter	I	*Klebsiella*	I
Actinobacillus	I	*Lactobacillus*	I
Actinomyces bovis	II	*Legionella*	II
eriksonii	II	*Leptospira*	II
israelii	II	*Leuconostoc*	I
naeslundii	II	*Listeria*	II
other spp.	I	*Microbacterium*	I
Aerococcus	I	*Micrococcus*	I
Aeromonas	I	*Moraxella*	I
Alcalescens	I	*Mycobacterium africanum*	III
Alkaligenes	I	*avium*	III
Arizona	I	*bovis*	III
Bacillus anthracis	II	*chelonei*	II
other spp.	I	*fortuitum*	II
Bacteroides	I	*intracellulare*	III
Bifidobacterium	I	*kansasii*	III
Bordetella	II	*leprae*	II
Borrelia	II	*marinum*	II
Branhamella	I	*malmoense*	III
Brevibacterium	I	*scrofulaceum*	III
Brochothrix	I	*simiae*	III
Brucella	III	*szulgai*	III
Campylobacter	I	*tuberculosis*	III
Chromobacterium	I	*ulcerans*	II
Citrobacterium	I	*xenopi*	III
Clostridium botulinum	III	other spp.	I
difficile	II	*Neisseria gonorrhoea*	II
fallax	II	*meningitidis*	II
novyi	II	other spp.	I
perfringens	II	*Nocardia*	II
septicum	II	*Pasteurella*	II
sordelli	II	*Pediococcus*	I
tetani	II	*Photobacterium*	I
other spp.	I	*Plesiomonas*	I
Corynebacterium diphtheriae	II	*Propionibacterium*	I
equi	I	*Proteus*	I
pyogenes	I	*Providence*	I
renale	I	*Pseudomonas mallei*	III
ulcerans	II	*pseudomallei*	III
other spp.	I	other spp.	I
Edwardsiella	I	*Salmonella paratyphi* A	III*
Eikenella	I	*typhi*	III*
Enterobacter	I	other serotypes	II
Erwinia	I	*Serratia*	I**
Erysipelothrix	II	*Shigella*	II
Escherichia	I	*Staphylococcus aureus*	II
Flavobacterium meningosepticum	I	other spp.	I
other spp.	I	*Streptobacillus*	II
Francisella	III	*Streptococcus* human and animal	
Fusobacterium	I	pathogens	II
Gardnerella	I	Food and milk spp.	I

(*continued overleaf*)

Table 3 (continued)

Organism	Risk Group	Organism	Risk Group
Streptomyces madurae	II	*Vibrio cholerae*	II
pelleteri	II	*parahaemolyticus*	I
somaliensis	II	other spp.	I
Treponema	II	*Yersinia pestis*	III
Veillonella	I	other spp.	I

*Require a Containment laboratory but not a safety cabinet; airborne infection unlikely
**Use a safety cabinet for experiments with aerosols.

Fungi and Yeasts

Organism	Risk Group	Organism	Risk Group
Acremonium	I	*Madurella*	II
Alternaria	I	*Microsporon*	II
Aspergillus	I♯	*Neurospora*	I
Blastomyces	II	*Paecilomyces*	I
Botrytis	I	*Paracoccidioides braziliensis*	II
Candida	I	*Penicillium*	I
Cladosporium	I	*Phialophora*	I
Coccidioides immitis	III	*Pichia*	I
Cryptococcus neoformans	II	*Pullularia*	I
Debaromyces	I	*Rhodotorula*	I
Endomyces	I	*Saccharomyces*	I
Epidermophyton	II	*Scopulariopsis*	I
Fonseceae	I	*Sporobolomyces*	I
Geotrichum	I	*Sporothrix*	II
Gliocladium	I	*Torulopsis*	I
Hansenula	I	*Trichoderma*	II
Helminthosporium	I	*Trichophyton*	II
Histoplasma capsulatum	III		
Kloeckera	I		

♯Use a safety cabinet for experiments which generate spores. These may be allergenic.

controlled ventilation), but reject the simpler and more effective methods (good technique).

Two examples of 'new' organisms are *Legionella* (and its associates) and the conjectural agent of acquired immune deficiency syndrome (AIDS). Others might be naturally occurring organisms suddenly elevated to prominence for industrial reasons, or even newly created organisms (although these should be adequately covered by genetic manipulation regulations). It is not unreasonable to place any such organisms in a high category or risk group until it is shown that it is safe to handle them without extra precautions. The trouble is that once an organism has been so placed it is very difficult, as in the case of hepatitis B virus, to bring it down to its proper level.

Pathogenicity tests

It may not be possible to determine the history of potential pathogenicity of an organism that has been found suitable for some laboratory or industrial

process. The question then arises: Can pathogenicity tests be done? This is another 'minefield'.

Laboratory animals have been used widely in medical microbiology to assess virulence or toxin production, but it is well known that some animals are immune to organisms which cause diseases in others, or in humans. If animal pathogenicity tests are to be used, it would become necessary to employ a fairly large number of different test species. A wide range of doses would also be needed, with and without steroids. Even then, it might be difficult to distinguish between specific pathogenicity, and intoxication or shock due to the injection of large amounts of foreign protein. It would also be necessary to use the various routes, oral and inhalation, as well as injection, to challenge different defence mechanisms. At the end of the experiment it would be difficult, in any case, to argue from some test animals, susceptible or not, that the organism is or is not pathogenic to man.

Some workers have turned to tissue culture tests but, as all virologists know only too well, some commensals quite harmless to man are cytotoxic. Whether an organism which fails to affect, say, human fibroblasts in tissue culture, is also incapable of causing human disease, is also open to conjecture. (I have grown tubercle bacilli in HeLa cells without apparently disturbing them.) This area of risk assessment is still very much in need of exploration.

Assessment of risk in genetic manipulation

Much time and labour has been devoted to this problem but, at present, any other than a very brief review should best appear in a paper dealing with the history of the subject. Changes may be expected.

Unlike risk assessment in conventional microbiology, it is the kind of experiments, not the micro-organisms, that are the centre of a classification scheme. Consideration is given to the source and purity of the nucleic acid used, the host/vector system, and the techniques employed. Certain factors are given probability values and the product of these values indicates the category of risk. The 'Access' factor measures the chances that the organisms will enter a human body and be able to initiate infection; the 'Expression' factor relates to the efficiency with which the foreign DNA is translated into protein; the 'Damage' factor the chance that a genetic element will cause damage, e.g. produce a toxin. This system gives four categories of risk (GMAG, 1978, 1981).

Assessment of risks in large-scale biotechnology

The production of amounts of 20 litres or of much greater volumes of natural or genetically manipulated organisms or their products can offer hazards to the health of the workers very similar to those from experiments conducted in a laboratory. Fortunately there are a number of reasons why larger-scale production is more likely to be safer than research.

Any hazards associated with the organisms, their possible mutants and their products, will already have been assessed in the research and development laboratory. Alternatively, the organisms will have been used in the industry for many years with no untoward incidents.

An 'intermediate technology' between the laboratory and the larger production plant has demonstrated that quite large volumes of even hazardous micro-organisms can be grown safely (Harris-Smith and Evans, 1968). Equipment such as they describe can be scaled up.

Many years of experience, accumulated by mechanical, hydraulic and electrical engineers in fermentation and similar industries, have given the products a high degree of protection from the environment. Those forms of protection can be adapted to protect the worker from the product. Examples of safety measures in downstream processing were presented at a recent meeting of the Society for Chemical Industry and the European Federation of Biotechnology (Lawrence and Barry, 1982; Turner, 1982; Walker and Foster, 1982; van Hemert, 1982).

Protection of the worker from infection (or allergy) is not the only consideration, however, in the assessment of risk. The possible effects on plant and animal life of effluents discharged into rivers or the sea, and of bacterial masses dumped on land, must also be considered, not only because the presence of living organisms may adversely affect the environment but because other economic or ecological 'undesirables' may flourish on their (living or dead) cells and their products.

Lastly, attention to hazards is the major task of health and safety authorities, and many processes involving micro-organisms are closely monitored by trade unions and conservationists.

Classification of laboratories for microbiology

Each of the three systems for classifying micro-organisms on the basis of hazards specifies laboratory facilities and precautions suitable for work within each risk group. These include physical containment in terms of building construction, ventilation, waste disposal and equipment; techniques used; personal precautions; and limitations on access. These all increase with increasing risk. In the American, but not the other systems, degrees of competence of staff are also specified. Although there are four risk groups (three in the UK), only three grades of laboratory are considered necessary. In the WHO (1979a, b) and USPHS (1983) classifications these are Basic, Containment and Maximum Containment, and correspond to the UK (DHSS, 1978) Categories C, B and A laboratories in order of containment. The Maximum Containment or Category A laboratories are sometimes referred to as Special Pathogens Units. The problem of fitting four Risk Groups of micro-organisms into three classes of laboratory was solved by the Americans (USPHS, 1983) by having four Biosafety Levels, which specify equipment and techniques. Two of these, Levels 1 and 2, apply in Basic laboratories according to whether the laboratory is used mainly for Risk Group I or for Risk Group II micro-organisms.

It would be tedious to reproduce here the precise requirements for each kind of laboratory and each Biosafety Level. They are therefore summarized below. For further information the references cited above and the appropriate national regulatory or advisory body should be consulted.

It is, of course, essential that all these laboratories are designed to meet the

requirements of the microbiologists who will be expected to work in them. It follows that these professionals should have a major voice in the design, not only because inept planning may directly influence the spread of airborne infection (Phillips, 1961; Collins, 1983), but to avoid any expensive alterations that might be required by the regulating authorities after the building is finished.

BASIC LABORATORIES (BIOSAFETY LEVELS 1 AND 2)

These are intended for work with organisms in Risk Groups I and II. Examples are college and quality control laboratories dealing with Risk Group I organisms (Biosafety Level 1); and clinical or diagnostic laboratories where many of the organisms encountered are in Risk Group II (Biosafety Level 2). No special building or engineering facilities are necessary at either level, but there should be hand-washing basins and also access to an autoclave so that all waste materials can be made safe. The principal hazards to be controlled, especially in clinical laboratories, are ingestion (e.g. by mouth pipetting) and injection. These call for an adequate level of technical competence. Similarly, the standards of hygiene, such as hand washing and wearing protective clothing, and also of access and vaccination, need to be maintained.

CONTAINMENT LABORATORIES (BIOSAFETY LEVEL 3)

These laboratories are necessary for work with organisms in Risk Group III which are most likely to infect laboratory workers by the airborne route or with relatively small doses by other routes. Special ventilation arrangements are therefore required to prevent the dispersal of infectious airborne particles into the room during manipulations and their transfer to other parts of the building. This is achieved by using microbiological safety cabinets (Classes I or II; *see below*) and maintaining pressure gradients so that air flows from the relatively 'clean' areas, e.g. Basic laboratories or corridors, into the Containment laboratories, and thence to atmosphere, filtered or otherwise according to local circumstances. This is not as difficult as some people believe and there are descriptions of simple systems (Clark, 1983; Collins, 1983). Access should be controlled so that members of the public and other unauthorized people cannot casually or inadvertently enter a Containment laboratory. The international Biohazard sign, with a cautionary notice for those who do not understand it, should be displayed on the doors of these laboratories. All equipment should be designed to minimize aerosol production and dispersal. An autoclave should be available.

A high standard of technical competence, again aimed at controlling aerosols, is essential, and the standards of hygiene and medical supervision should be higher than those specified for work in Basic laboratories. Appropriate vaccinations should be mandatory.

MAXIMUM CONTAINMENT LABORATORIES (BIOSAFETY LEVEL 4)

These are essential for work with Risk Group IV pathogens which offer serious

'life-threatening' hazards to the individual worker and the community. In most countries there are laws or regulations about the construction and operation of these laboratories.

Sophisticated engineering facilities are essential to control air flows and to ensure negative pressure gradients in the rooms, the filtration of effluent air and the decontamination, by steam or chemicals, of all liquid effluents and sewage. Physical separation of the laboratory rooms from the same or nearby buildings is required, with access and egress through airlocks and showers. Access is strictly controlled. Infectious material is taken in via a separate airlock and is processed in Class III cabinets or negative-pressure flexible-film isolators which contain centrifuges, incubators and all other equipment. Alternatively, the work is done on the open bench with the operators enclosed in positive-pressure flexible suits with an external air supply from flexible 'umbilical' tubes. All discarded material leaves through a double-doored autoclave with safety locks so that the outer door can be opened only after a sterilization cycle is complete. Very high standards of technical competence, personal protection and medical supervision are essential, as is specific vaccination if available. When work is in progress at least two persons should be present.

Classification of laboratories for genetic manipulation

The principles are the same as those outlined above for conventional microbiology but there are four grades of laboratories, corresponding to four categories of risk. In the UK these are designated Categories I-IV in ascending order of containment (GMAG, 1981), and in the US P1–P4 where P means Physical Containment (USPHS, 1978). The two systems are roughly comparable. Genetic manipulation laboratories are subject to inspection by regulatory bodies and in the UK at present Categories III and IV laboratories are visited by the Genetic Manipulation Advisory Group.

CATEGORY I

This is a Basic Laboratory which must have a hand-basin, and a Class I safety cabinet if any of the work is likely to generate aerosols. An autoclave must be available and all waste material rendered safe before disposal. The laboratory should not, as far as possible, be used for other purposes.

CATEGORY II

The requirements for this kind of laboratory are almost the same as those for a Containment Laboratory for conventional microbiology: designation for a specific purpose; air lock; restricted access; continuous airflow from 'clean' to 'dirty' areas and thence to atmosphere through a HEPA filter; a Class I microbiological safety cabinet and an autoclave in the laboratory or nearby.

CATEGORY III

This is somewhere between the Containment and a Maximum Containment laboratory used for conventional microbiology. The laboratory must be physically isolated, with controlled access and not near to such hazardous areas as solvent stores; it should not be liable to flooding. Entry is through an airlock. A negative pressure gradient is necessary and exhaust air must be passed through a HEPA filter before dispersal to atmosphere. An autoclave must be provided, either in an adjoining room and used solely for Category III work, or double-ended with appropriate safeguards. Effluents must be made safe before disposal. One or more Class III microbiological safety cabinets are required. There are also requirements for protective clothing, respirators, lockable refrigerators and telephones.

CATEGORY IV

This is a full Maximum Containment laboratory and, in addition to all the requirements of a Category III laboratory, there must be showers between the clean side of the airlock and the laboratory, and an emergency electric power generator that automatically operates if the main power source fails.

The containment requirements for genetic manipulations are therefore slightly different from those for conventional microbiology. It is unfortunate that these two systems are not in step. This can cause irritation, and even problems and confusion in establishments (and, at the lower level, even rooms) where the two activities coexist. Now that we have a new broom in the form of ACDP, and GMAG may well become reconstituted, it should be possible to equate the two laboratory classifications and containment requirements.

Microbiological safety cabinets

These very important pieces of containment equipment are mentioned above and merit a note here. For more detailed information about design, siting, installation, testing and use, the current British Standard (BS 5726: 1979) and the books by Clark (1983) and Collins (1983) should be consulted.

There are three Classes, I, II and III, but these numbers bear little relation to the degree of protection afforded. Classes I and III belong to the same genus; Class II works on an entirely different principle.

CLASS I

This is shown diagrammatically in *Figure 1a*. The operator works with bare or gloved hands in the centre or rear of the cabinet while his face is protected by the glass screen. Room air is pulled in through the working face, past the operator's arms at a velocity (0·75–1·0 m/s at the face) calculated to entrain any aerosols and prevent their escape into the room. Aerosols and airborne particles are removed by a coarse filter and a High Efficiency Particulate Air (HEPA) filter; the clean effluent is exhausted outside the building.

Class I cabinets are suitable for work with most micro-organisms up to and including those in Risk Group III, and are intended to protect the operator.

CLASS II

One of these is shown in *Figure 1b*. The operator works and observes as in a Class I cabinet. Air is re-circulated round the cabinet by an integral fan, passing through a HEPA filter before descending through the working space at 0·4–0·5 m/s, and returning through grilles in the floor and rear of the working area. The vertical curtain of air at the working face acts as a barrier to prevent contaminants entering the cabinet from the room. About 30% of the recirculated air is exhausted to atmosphere and is replaced by room air which enters through a grille in the aerofoil at the front of the cabinet floor. This additional curtain prevents the escape of organisms into the room.

Class II cabinets are intended to protect both the worker and the work and are thus particularly useful for handling tissue cultures with organisms up to and including those in Risk Group III. There is an official prejudice against them in UK clinical laboratories because the Howie Working Party (DHSS, 1978) considered that some of the cabinets available at that time did not provide enough protection to the worker. Since then, however, cabinets made to the British Standard (1979) and, in America, to the National Sanitation Foundation Standard (1976) have become available and these do offer adequate operator protection. Class II cabinets are extensively used in the US, where Class I cabinets are unpopular, and there is no evidence that they have failed to protect laboratory workers from infection. They are more expensive, however, to purchase, install and maintain than are Class I cabinets. It is of interest that the amended GMAG Code of Practice (GMAG 1981) permits Class II cabinets to be used for certain procedures with tissue cultures 'which may produce only small amounts of aerosol'.

CLASS III

A Class III cabinet is shown in *Figure 1c*. It resembles a Class I cabinet but the front is closed and sealed. The cabinet is gas-tight. The operator works with gloves, sealed into glove ports. Air enters through one HEPA filter and is exhausted to atmosphere through at least one other. Materials and equipment may be loaded, before work is started, by unclamping the front, or loaded and removed through side ports with air locks or dunk tanks. These also allow the cabinets to be connected in series.

FLEXIBLE-FILM ISOLATOR

This is a modification for laboratory use of the patient isolator developed by Trexler (van der Groen, Trexler and Pattyn, 1980; *see also* Collins and Yates, 1982). A heavy-duty plastic envelope, fitted with glove ports, is mounted on a trolley and maintained at a negative pressure relative to the room by an exhaust fan and valves. Air enters through one HEPA filter and is ducted to atmosphere

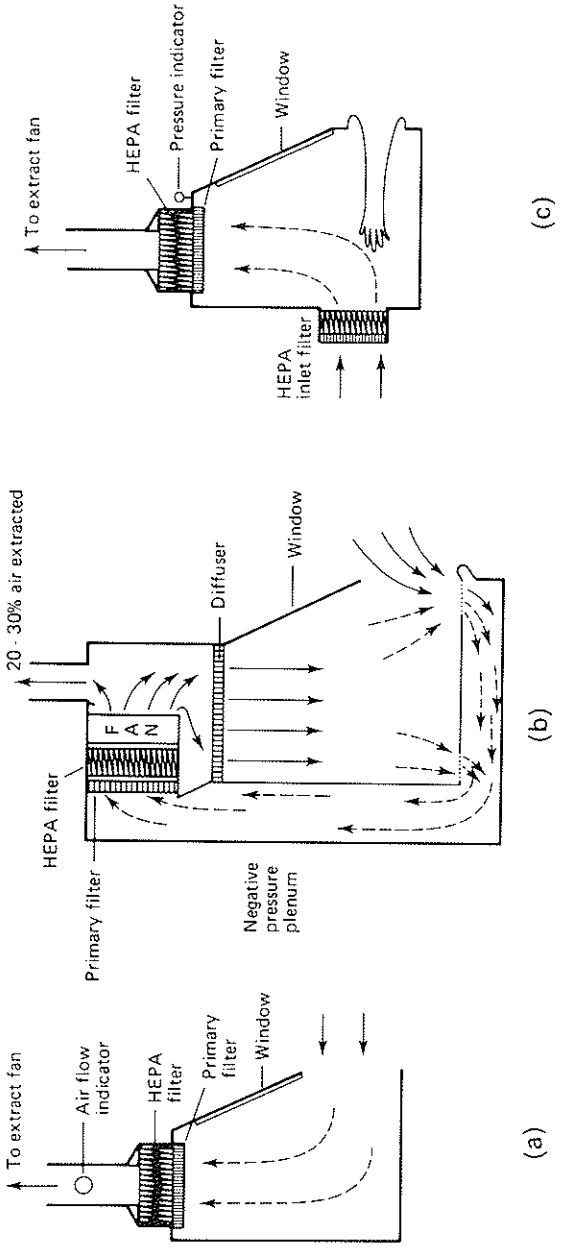

Figure 1. Microbiological safety cabinets showing air flows and general features. (a) Class I. (b) Class II. (c) Class III (from Collins, 1983, by courtesy of Butterworths Ltd). ——— Clean air – – – – Contaminated air.

through another. The isolator is mobile, with ports which enable materials to be 'bagged' in and out in plastic bags. It can be docked with other isolators including those accommodating patients. There is space for quite large equipment, with air-tight service connections. As it is double-sided at least four people can work at the same time.

Conclusions

It is quite impossible for anyone who has studied the history of laboratory-associated infections to conclude that no hazards exist in work with micro-organisms. Only those who are unaware of the facts, and are not themselves at risk, will dismiss these hazards as acceptable or non-existent.

At the same time, such studies will confirm that all, or very nearly all, of these hazards have been identified and that means exist to neutralize or overcome them. The paradox is that although little or no interest or action was taken in the well-documented risks in conventional microbiology (until the smallpox incidents described on p. 143, 144), a great deal of concern has been directed at the conjectural hazards of genetic manipulation. But for the smallpox episodes, codes of practice and regulations for genetic engineering would have antedated those for microbiology, probably by many years. Nevertheless, although the means to deal with the hazards are mostly within the power and competence of the scientists themselves, the laboratory workers can cope properly only if they have adequate and suitable accommodation, proper equipment and good training facilities.

In the interests of public confidence, laboratory workers also need codes of practice and advisory and regulatory bodies, as much to protect themselves as to protect the general public, and to ensure that resources are directed where they are most needed. This, of course, means committees, and in this age of decision-taking by committees, all vested (and some unvested) interests will fight to have representation. Some of the representatives on these committees may not be well informed and may lack practical experience, and these may even outnumber the practising scientists. There is also the danger that these committees may become self-perpetuating bureaucracies serving no purpose but their own. There seems to be a belief that scientists cannot be trusted, but there is no evidence that decisions made by lay persons about, say, containing a newly discovered pathogen such as *Legionella*, or a postulated one such as the agent of AIDS, are likely to be more correct than those of scientists. Scientists are, generally, responsible citizens, taxpayers and (usually) trade unionists.

References

ANONYMOUS (1979). Laboratory-associated typhoid fever. *Morbidity and Mortality Weekly Reports* **28,** 44.

BANERJEE, K., GUPTA, N.P. AND GOVERDHAN, M.K. (1979). Viral infections in laboratory personnel. *Indian Journal of Medical Research* **69,** 363–373.

BERG, P., BALTIMORE, D., BOYER, H.W., COHEN, S.N., DAVIS, R.W., HOGNESS, D.S., NATHANS, D., ROBLIN, R.O., WATSON, J.D., WEISSMAN, S. AND ZINDER, N.D. (1974). Potential biohazards of recombinant DNA molecules. *Science* **185,** 303.

BERG, P., BALTIMORE, D., BRENNER, S.O., ROBLIN, R.O. AND SINGER, M.F. (1975), Summary statement of the Azilomar Conference on recombinant DNA molecules. *Science* **188,** 991, and *Nature* **225,** 442.

BRITISH STANDARD (1979). *Specification for Microbiological Safety Cabinets.* BS 5726. British Standards Institution, London.

CLARK, R.P. (1983). *The Performance, Installation, Testing and Limitation of Microbiological Safety Cabinets.* Occupational Hygiene Monograph No. 9. Science Reviews, Northwood.

COHEN, D.R. (1982). The Howie Code: is the price of safety too high? *Journal of Clinical Pathology* **35,** 1018–1023.

COLLINS, C.H. (1983). *Laboratory-Acquired Infections: History, Incidence, Causes and Prevention.* Butterworths, London.

COLLINS, C.H. AND LYNE, P.M. (1984). *Microbiological Methods,* 5th edn, chapter 1. Butterworths, London.

COLLINS, C.H. AND YATES, M.D. (1982). The use of a flexible film isolator in a diagnostic laboratory. *Laboratory Practice,* September 1982, 892–894.

DE KRUIF, P. (1926). *The Microbe Hunters.* Jonathan Cape, London.

DE KRUIF, P. (1933). *Men Against Death.* Jonathan Cape, London.

DEPARTMENT OF HEALTH AND SOCIAL SECURITY (1976). *Control of Laboratory Use of Pathogens very Dangerous to Humans.* Department of Health & Social Security, London.

DEPARTMENT OF HEALTH AND SOCIAL SECURITY (1978). *Code of Practice for the Prevention of Infection in Clinical Laboratories and Post-mortem Rooms.* HMSO, London.

GENETIC MANIPULATION ADVISORY GROUP (1978). Genetic manipulations: new guidelines for UK. *Nature* **276,** 104–108.

GENETIC MANIPULATION ADVISORY GROUP (1981). *Code of Practice for Genetic Manipulation: Containment Facilities.* Medical Research Council, London.

GRIST, N.R. (1975). Hepatitis in clinical laboratories; a three year survey. *Journal of Clinical Pathology* **28,** 255–259.

GRIST, N.R. (1976). Hepatitis in clinical laboratories 1973–1974. *Journal of Clinical Pathology* **29,** 480–483.

GRIST, N.R. (1978). Hepatitis in clinical laboratories 1975–1976. *Journal of Clinical Pathology* **31,** 415–417.

GRIST, N.R. (1980). Hepatitis in clinical laboratories 1977–1978. *Journal of Clinical Pathology* **33,** 471–473.

GRIST, N.R. (1981). Hepatitis and other infections in clinical laboratory staff. *Journal of Clinical Pathology* **34,** 655–658.

GRIST, N. (1983). Infections in British clinical laboratories 1980–81. *Journal of Clinical Pathology* **36,** 121–126.

HARRINGTON, J.M. AND SHANNON, H.S. (1976). Incidence of tuberculosis, hepatitis, brucellosis and shigellosis in British medical laboratory workers. *British Medical Journal* **1,** 759–762.

HARRIS-SMITH, R. AND EVANS, C.G.T. (1968). The Porton mobile enclosed chemostat (POMEC) In *Continuous Cultivation of Micro-organisms.* Proceedings of 4th Symposium. Academia, Prague.

HUNTER, D. (1936). Saints and martyrs. *Lancet* **ii,** 1131–1134.

KISSKALT, K. (1929). Laboratoriumsinfectionen mit Typhuzbazillen und anderen Bakterium. *Archiv für Hygiene und Bakteriologie* **101,** 137–160.

LAWRENCE, A. AND BARRY, A. (1982). Potential hazards associated with the large-scale manufacture of bacterial vaccines. *Chemistry and Industry* 20 November, 880–884.

LUBARSCH, O. (1931). *Ein bewegtes Gelehrtenleben,* p. 68. Springer, Berlin.

MICROBIOLOGICAL CONSULTATIVE COMMITTEE (1980, 1982). *Guidelines for Microbiological Safety.* Society for General Microbiology, Reading.

NATIONAL SANITATION FOUNDATION (1976). *Standard No.* 49. *Class II (Laminar Flow) Biohazard Cabinetry.* Ann Arbor, Michigan.

PANETH, L. (1915). The prevention of laboratory infections. *Medizinische Klinik* **11,** 1398–1399.

PAPP, K. (1959). The eye as a portal of entry of infections. *Bulletin of Hygiene* **34,** 969.

PHILLIPS, G.B. (1961). *Microbiological Safety in US and Foreign Laboratories. Technical Report No.* 35. *US Army Chemical Corps.* US Army, Washington.

PHILLIPS, G.B. (1969). Control of microbiological hazards in the laboratory. *American Industrial Hygiene Association Journal,* **30,** 170–176.

PIKE, R.M. (1976). Laboratory associated infections. Summary and analysis of 3921 cases. *Health Laboratory Science* **13,** 105–113.

PIKE, R.M. (1978). Past and present hazards of working with infectious materials. *Archives of Pathology and Laboratory Medicine* **102,** 333–336.

PIKE, R.M. (1979). Laboratory associated infections; incidents, fatalities, causes and prevention. *Annual Review of Microbiology* **33,** 41–66.

PIKE, R.M., SULKIN S.E. AND SCHULZE, M.L. (1965). Continuing importance of laboratory acquired infections. *American Journal of Public Health* **55,** 190–199.

REID D.D. (1957). The incidence of tuberculosis among workers in medical laboratories. *British Medical Journal* **ii,** 10–14.

REPORT (1974). *Report of the Committee of Inquiry into the Smallpox Outbreak in London, March–April,* 1973 (*Chairman P.J. Cox*). Cmnd. 5626. HMSO, London.

REPORT (1975a). *Report of the Working Party on the Laboratory Use of Dangerous Pathogens* (*Chairman Sir George Godber*). Cmnd. 6054. HMSO, London.

REPORT (1975b). *Report of the Working Party on the Experimental Manipulation of the Genetic Composition of Micro-organisms* (*Chairman Lord Ashby*). Cmnd. 5880. HMSO, London.

REPORT (1976). *Report of the Working Party on the Practice of Genetic Manipulation* (*Chairman Sir Robert Williams*). Cmnd. 5880. HMSO, London.

REPORT (1980). *Report of the Inquiry into the Causes of the* 1978 *Birmingham Smallpox Occurrence* (*Chairman R.A. Shooter*). House of Commons Paper 79–80. HMSO, London.

REPORT (1983). *Report No.* 1 *of the Advisory Committee on Dangerous Pathogens: Categorisation of Pathogens According to Risk and Categories of Containment* (*Chairman D.A.J. Tyrrell*). Department of Health and Social Security and the Health and Safety Executive, London.

SULKIN, S.E. (1961). Laboratory acquired infections. *Bacteriological Reviews* **25,** 203–209.

SULKIN, S.E. AND PIKE, R.M. (1951). Laboratory acquired infections. *Journal of the American Medical Association* **147,** 1740–1745.

TURNER, M. (1982). Downstream processing: hygiene or containment? *Chemistry and Industry,* 20 November, p. 876.

UNITED STATES PUBLIC HEALTH SERVICE (1974a). *Classification of Etiologic Agents on the Basis of Hazard.* Centers for Disease Control. Government Printing Office, Washington.

UNITED STATES PUBLIC HEALTH SERVICE (1974b). *National Institutes of Health Biosafety Guide.* Government Printing Office, Washington.

UNITED STATES PUBLIC HEALTH SERVICE (1974c). *Lab Safety at the Center for Disease Control.* Government Printing Office, Washington.

UNITED STATES PUBLIC HEALTH SERVICE (1976). National Institutes of Health. Recombinant DNA Research: Guidelines. *Federal Register* **41,** No. 131, Part II (July).

UNITED STATES PUBLIC HEALTH SERVICE (1978). *National Institutes of Health Laboratory Safety Monograph. Supplement to the NIH Guidelines on Recombinant DNA Research.* Government Printing Office, Washington.

UNITED STATES PUBLIC HEALTH SERVICE (1980a). National Institutes of Health: Guidelines for Research Involving Recombinant DNA Molecules. *Federal Register* **45,** No. 20, Parts V and VI (January).

UNITED STATES PUBLIC HEALTH SERVICE (1980b). *Proposed Biosafety Guidelines for Biomedical and Microbiological Laboratories.* Centers for Disease Control, Atlanta.

UNITED STATES PUBLIC HEALTH SERVICE (1981). Classification of Etiologic Agents on the Basis of Hazard. *Federal Register* **46,** 59379–59380.

UNITED STATES PUBLIC HEALTH SERVICE (1983). *Biosafety in Microbiological and Biomedical Laboratories.* Centers for Disease Control, Atlanta; National Institutes of Health, Bethesda.

VAN DER GROEN, G., TREXLER, P.C. AND PATTYN, S.R. (1980). Negative pressure flexible film isolator for work with Class IV viruses in a maximum security laboratory. *Journal of Infection* **2,** 165–170.

VAN HEMERT, P. (1982). Biosafety aspects of a closed system Westfalia type continuous flow centrifuge. *Chemistry and Industry* 20 November, 889–891.

WALKER, P.D. AND FOSTER, W.H. (1982). Containment in vaccine production. *Chemistry and Industry*, 20 November, 884–887.

WILLIAMS, R.E.O. (1981). In pursuit of safety. *Journal of Clinical Pathology* **34,** 232–239.

WORLD HEALTH ORGANIZATION (1979a). Safety measures in microbiology; the development of emergency services. *Weekly Epidemiological Record* No. 20, 154–156.

WORLD HEALTH ORGANIZATION (1979b). Safety measures in microbiology; minimum standards of laboratory safety. *Weekly Epidemiological Record* No. 44, 340–342.

WORLD HEALTH ORGANIZATION (1980). Guidelines for the management of accidents involving micro-organisms. A WHO Memorandum. *Bulletin of the World Health Organization* **58,** 245–256.

WORLD HEALTH ORGANIZATION (1983) *Laboratory Biosafety Manual.* WHO, Geneva.