

SCOTTISH SCHOOLS SCIENCE

EQUIPMENT RESEARCH

CENTRE

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# Introduction

This bulletin is wholly concerned with microbiological hazards. It contains the best and most up to date advice we can give on the subject and the material has been widely researched. Because microbiology is not confined to science classes, we believe that others who include primary schools advisers, teachers of anatomy, physiology and health, home economics and perhaps physical education should have access to the advice we give. Hence we have printed additional copies of this issue which may be obtained from SSSERC by anyone in the educational field who intends doing, or advising on school microbiology. Copies will cost 20p each, or 15p to callers at the Centre.

In addition, the staff at SSSERC are prepared to come and talk to groups of teachers on the subject, and to demonstrate safe microbiological techniques. One such lecture which, although it is a general one on safety, will obviously include microbiology, has already been arranged by the Central Region science teachers association and will take place in Falkirk on 28th September.

## Biology Notes

Rarely is microbiological work in schools concerned with known pathogenic (disease-causing) organisms. In addition the human body has a battery of immunological and other weapons to help ward off infection. However, safety measures in school microbiology are essential for several reasons:

(a) The dividing line between pathogens and non-pathogens is not clear cut. The blurred area between them may never be clear, but it has extended with time. A number of school texts (1) have recommended the routine use of 'harmless' organisms which we now recognise as potential human pathogens.

(b) The possibility of chance contamination by pathogens exists. Culturing procedures designed to concentrate relatively harmless organisms for study can also concentrate pathogens that normally occur in the environment at sub-infective levels.

(c) The properties of micro-organisms are not fixed. Mutation is a rare but continuing phenomenon. Non-pathogenic strains can mutate and become potentially harmful. Exposure of certain organisms to antibiotics, either deliberately in an experiment, or accidentally because laboratory workers or pupils are undergoing medical treatment can lead to the development of resistance. The resistant strains can then cause illness, either directly or by transferring their resistance to other, pathogenic, bacteria.

Medical journals contain scattered reports of relatively 'harmless' saprophytes becoming pathogenic. Some reporters have observed

that such occurrences have become commoner since the introduction of the sulphonamide drugs and antibiotics. The bacteria involved include some hitherto used in school microbiological work: Serratia marcescens; Pseudomonas aeruginosa; and Chromobacterium violaceum. The organisms involved also usually show considerable resistance to the commoner antibiotics (2, 3) and fatalities have occurred despite intensive therapy.

In any potentially hazardous situation, the dangers must first be recognised before they can be controlled or eliminated. This requires a thorough knowledge and adequate training. The biggest microbiological hazard arises from ignorance of the inherent dangers in any work designed to concentrate micro-organisms. Under the Health and Safety at Work Act 1974, as in other aspects of their work, employers and teachers must ensure that 'everything reasonably practicable' has been done to reduce hazards to a minimum.

In school microbiology there are three levels of work which can be identified:

(a) Work with organisms which can be used with little if any risk, where faulty technique may lead to the demise of the cultures but is unlikely to put pupils or teachers at risk, e.g. with algae, non-pathogenic yeasts or moulds and protozoa.

(b) Work where prepared media are exposed to the environment or are inoculated with material from the environment. Isolates are not made and cultures are not transferred. Consequently only rudimentary aseptic technique is required. Providing certain precautions are observed, non-specialist staff and secondary school pupils may safely carry out this work.

(c) Work with cultures of fungi and bacteria where subculturing and transfer procedures are required as a matter of routine. Good aseptic technique is required and this can only be acquired after thorough training and practice. This work would not normally be undertaken or supervised by non-specialist staff unless they have attended a suitable course of in-service training.

Little more needs to be said about work at level (a). Culture media are used but are generally of a type not likely to encourage growth of pathogens. All micro-organisms should be treated with caution and elementary rules of hygiene should still be observed. Some authors recommend the use of faecal material as an 'enrichment' in the preparation of infusoria etc. These recommendations should be ignored.

Work at level (b) is potentially hazardous but the risk to pupils and staff should be minimal if certain elementary precautions are taken. In school courses this level of work usually involves exposing prepared plates of nutrient agar to various sources of micro-organisms. If class discipline cannot be relied on, consideration should be given to restricting this level of work to teacher demonstration. This is a regrettable but, with some pupils, necessary consideration.

The essential difference between this work and level (c) lies in the fact that microbial growths are not subcultured after incubation. The growths produced after exposure and incubation are merely observed and recorded, after which they are destroyed. When doing this work the following safety precautions should be observed

(4, 5, 6, 7, 8):

(I) Before starting work, wash the hands and dress cuts with waterproof dressings. Before leaving the laboratory hands should be washed with soap and hot water.

(II) Avoid culturing microbes from potential sources of pathogens such as human (or animal) mucus, pus from cuts or faecal material.

(III) Natural sources such as soil, water and raw milk can all contain pathogens. As a general rule ALL PLATES SHOULD BE SEALED DURING AND AFTER INCUBATION. If plates are fully sealed by taping round their circumference, growth may be atypical because of reduced oxygen content. Apparently some tapes are bactericidal (8) and may prevent growth during incubation. It is probably better to use several smaller pieces of tape, at the sides of dishes, holding together the base and lid.

(IV) Petri-dish cultures should be incubated base uppermost so that any condensation will drip into the lid. The cultures should be properly labelled either with a wax pencil or a self-adhesive label.

(V) If a great deal of condensation occurs, making it difficult to see the culture, a replacement dry, sterile lid should be fitted by the teacher or technician, and the dish resealed. Replacement of lids should be rapid, but careful, since aerosols may be formed. The new lid should be immediately to hand when the old lid is removed. Throughout the operation the lid should be held open side downwards and the transfer carried out over a piece of lint moistened with a suitable disinfectant (see page 7). The old lid should be disposed of by the methods described below.

(VI) Inspection of open culture dishes by pupils is rarely if ever necessary at this level. If it is allowed, the micro-organisms should be killed by placing a filter paper moistened with a few drops of 40 per cent methanal solution (care) in the dish 24 hours prior to examination. It is our opinion that culture plates which have been used in any experiment involving antibiotics should be kept sealed at all times and should be disposed of unopened.

(VII) All hand to mouth operations should be avoided. There should be no smoking, eating or drinking in the laboratory. Self-adhesive labels or wax pencils should be used but not the gummed type of label.

(VIII) Cultures should be incubated at an appropriate temperature. Incubation at 37°C, a temperature recommended by many authors for general bacteriological work, tends to select organisms adapted to man's body temperature. Incubation at high temperatures can isolate thermophilic fungi some of which produce infective spores. In elementary work, anaerobic culture should be avoided since there is a danger of isolating anaerobic, enteric and other pathogens. The use of certain enrichment media such as blood agar, bile salts etc. is also inadvisable at this level because it increases the chances of isolating pathogenic organisms.

(IX) Microbiological materials should never be kept in

refrigerators used for home economics etc. Similarly it is part of good laboratory practice that food for human consumption should never be kept in laboratory refrigerators.

(X) Pupils should realise that all contaminated equipment must first be sterilised even if it can be washed and re-used. Special areas or containers should be set aside for contaminated apparatus and materials. Spillages of contaminated material on bench, floor or person must be reported to the teacher and swabbed with a suitable disinfectant (see page 7).

(XI) No experiment should be performed which involves deliberate contamination of the skin or other parts of the bodies of pupils.

At level (c) inoculation, sub-culturing, dilution plating and other procedures may be carried out as routine techniques. These procedures involve a large number of operations where microbial aerosols may be formed. These aerosols consist of finely disseminated droplets and particles and can be formed whenever the surface of a liquid is broken or when dry material is crushed, ground or shaken. Once formed they may spread extensively, causing widespread contamination and may persist in the laboratory for some time. The particles are small enough to penetrate deep into the respiratory passages where what would normally be a sub-infective dose can cause illness. Bacterial and fungal spores are ubiquitous. Unless technique is good, experiments can be ruined and far worse, chance contamination by, and isolation of pathogens may occur. The importance of adequate theoretical and practical training should not be underestimated. Anyone carrying out work at this level should adhere strictly to the correct methods of handling cultures and apparatus until these become habitual. Good aseptic technique is of paramount importance and in addition to the safety precautions outlined for level (b) the following points should be noted:

(I) The work area should be away from open windows, doors and other sources of draughts. A germicidal aerosol, 70 per cent ethanol: 30 per cent water (fire hazard) or a 1 per cent solution of an ampholytic surfactant such as 'Tego MHG', Gerrard ASAB or Harris BAS is sprayed into the air immediately above the work area. Droplets are allowed to settle, the bench swabbed and the work started.

(II) If a good deal of transfer work, sub-culturing, plating out etc. is done, it may be worthwhile constructing or buying a transfer chamber. The chambers on the schools market do help to cut down the general level of contamination in the laboratory. However they are not designed to afford protection against potential pathogens. The use of such a chamber does not protect the operator against the consequences of poor technique.

(III) Work close to a bunsen burner so that organisms are prevented from falling into apparatus by the updraught.

(IV) A good deal of technique is associated with the use of the inoculating loop. Inoculating loops can be easily made by bending 24 s.w.g. nichrome wire round a match stick or by using round-nosed pliers. Such loops are sometimes attached to soda glass rods but a much better holder is the metal chuck type. This is because flame sterilisation should include the lower part of the handle. When this is attempted with glass loop holders they

often shatter. A few straight wires should be available for making stab cultures. For fungal work a stouter wire, with a flattened end in the form of a blade, is often used for cutting out pieces of agar and mycelia. Some workers use a wire with a right angle bend at the tip for transferring fungal spores.

(V) Any instrument introduced into a culture must first be sterilised. Wire inoculating loops are heated to red heat by passing the loop, and the lower part of its holder, through a roaring bunsen flame. The wire should be allowed to cool before use on a culture plate, or can be quenched in sterile water in a sterile Petri dish before use. Alternatively the wire can be dipped in ethanol and passed through the flame to burn off any excess. Direct flaming of a wet loop can cause sputtering, and material which spits from an overcharged loop may not have been sterilised. In this situation the formation of a contaminated aerosol is likely. This can be avoided by immersing the contaminated loop in a beaker of boiling water before flaming. Special safety incinerators are available which contain and sterilise any material which spits from loops, but these are somewhat expensive.

(VI) Flame the apertures of glassware (culture tubes, McCartney bottles, flasks etc.) after removing caps or plugs, before use and repeat the flaming before replacement. Plugs and caps should not be placed on the bench. With practice it is possible to manipulate tubes, plugs and inoculating loop without any of them leaving the hands. However, if tubes or caps cannot be handled conveniently they may be placed on a clean surface, such as a ceramic tile which has been swabbed with a suitable disinfectant. Always support culture tubes and similar glassware in a rack, preferably a plastic coated wire one, never prop them up or lay them down on the bench.

(VII) Lids of Petri dishes should be opened just enough to allow the inoculating tool to enter and be manipulated. Lids should be opened for the minimal amount of time necessary for the particular operation to be performed. The lid should be held open at an angle and the opening should face away from the worker.

(VIII) Glassware such as pipettes is best sterilised by dry heat in an oven at 140 - 180°C for at least an hour. The pipettes are wrapped in paper or aluminium foil and placed in a metal pipette canister. It is essential to allow a warm up period for the oven and contents. Square pipette canisters are preferable to round ones which can roll when placed on the bench (8).

(IX) Chemical disinfection should be reserved for the treatment of spillages and contaminated, used equipment. It should not be used for the preparation of equipment, since it tends to be slow and is only completely reliable on a commercial scale (8). Ultra-violet lamps are fitted in some transfer chambers where they are used for surface and background air sterilisation. However u.v. is not appropriate for general sterilisation of glassware or other equipment. It will not penetrate glass, films of dirt on organisms or on the surface of the lamp itself. U.V. lamps will continue to emit visible radiation after emissions at the germicidal wavelength have ceased (7).

(X) Liquids and articles which would be damaged by dry heat at 140 - 180°C are sterilised by steam at 120°C for 15 minutes (103 kN/m<sup>2</sup> or 15 lbs/in<sup>2</sup> steam pressure). This can be done in a

small autoclave or domestic pressure cooker. During autoclaving, lids of bottles must be left slightly loose to allow for changes of pressure. A pressure cooker should not be cooled too quickly or liquids may boil over and be wasted. If an autoclave proper is used then the following safety precautions should be observed:

- (a) fill to the correct level with water.
- (b) avoid overloading and take care not to block openings to pressure gauge or safety valve. All air should be expelled before closing the steam valve.
- (c) after the required autoclaving period, release the steam outlet valve and allow the pressure to drop to atmospheric before opening the door. Do not rely solely on any pressure gauge fitted to the device.
- (d) allow the contents to cool before removing them or wear heavy heat resistant leather gloves.

(XI) Pipettes, including Pasteur pipettes, have their wide end plugged with cotton wool in an attempt to keep the interior dust and microbe free. Plugs do not offer an effective barrier to the passage of liquids and they are easily penetrated by organisms in liquid suspension. If plugs become wet they obstruct air flow and can be readily sucked out followed by an unimpeded gush of liquid. For this reason, pipettes are never used in the mouth. A variety of rubber bulbs, 'pi-pumps' and other devices are available to draw fluid into the lumen of the pipette. For quantitative pipetting either sterile disposable syringes or graduated glass pipettes, heat sterilised in aluminium foil and operated by an autoclavable rubber bulb or similar device should be used. Aerosols formed by accidental spillage from pipettes etc. can be reduced by carrying out operations over a piece of lint moistened with a suitable disinfectant. The lint should only be moist, when fallen drops will immediately diffuse into it. If it is saturated then an aerosol may still be produced (4).

(XII) Where 'stock' cultures are kept they should be checked for contamination before being used in any experiments. A sub-culture should be plated out and examined for any mixed growth. If mixed cultures are found the stock should be destroyed by autoclaving and a fresh culture bought in (5).

#### Treatment of contaminated equipment.

The procedures detailed here should be followed with all contaminated equipment whatever the level of investigation.

(I) The safest disposal procedure is to use autoclavable disposal bags. Plastic materials such as disposable Petri dishes should be placed in the bag which is then sealed and autoclaved. The sterilised material should then, ideally, be incinerated but may, as a second best, be passed into the normal refuse disposal system. Retainable glassware can be autoclaved directly and then washed and stored. Before re-use such glassware should be sterilised by autoclaving.

(II) If the only autoclave available is a domestic pressure cooker, then disposal of a large amount of material can mean having to autoclave in several batches. This can be extremely



tedious and it is sometimes suggested (5) that in these circumstances culture plates etc. can be placed in a bucket of disinfectant and left to soak, at least overnight, before final disposal. It must be stressed that this is a less safe procedure than autoclaving and is best not adopted as the routine method. Chemical disinfection before disposal should use the disinfectants specified below for pipettes.

(III) Contaminated pipettes should be placed in a container with a suitable disinfectant, i.e. those relying on available chlorine or an ampholytic surfactant for their action. Solutions of the former type should contain at least 200 ppm available chlorine and in dirty conditions up to 1000 ppm may be required. They may be made up using sodium chlorate (I) (hypochlorite), 'Chloros', 'Milton' or a domestic bleach. Because the concentration of chlorate (I) varies in proprietary preparations, the manufacturers' instructions should be followed. Chlorate (I) solutions are corrosive and irritating, so that protective gloves should be worn and solutions kept off any metallic parts of apparatus. As they give off their chlorine readily and so diminish in effectiveness, the solutions should be made up freshly each time they are to be used. Starch iodide papers turn deep blue in solutions with 200 ppm available chlorine and can be used to check the disinfectant for effectiveness (4). 'Tego MHG', Gerrard ASAB and Harris BAS are ampholytic surfactants and can be used in 1% solution as alternatives to 'chlorine' (9).

The pipettes are soaked in the disinfectant solution overnight and, ideally, are then autoclaved together with the pipette pot in which they have soaked. They are then washed, placed in suitable containers and sterilised by dry heat before being used again. Some graduated pipettes are too large to fit in the domestic pressure cookers used in many schools. These larger pipettes will have to be washed immediately after soaking overnight in disinfectant and sterilised directly by dry heat. Lysol and other solutions containing the lower phenolics are toxic, caustic and non-sporicidal, and should not be used in schools (9).

(IV) Spillage on laboratory benches, floors etc. should be immediately swabbed with a concentrated chlorate (I) solution. As in (III) protective gloves should be worn. Because these solutions will bleach clothing and corrode metal, spillages on the person and on apparatus should be swabbed with an ampholytic surfactant or quaternary ammonium compound disinfectant.

(V) Many laboratory sinks have open traps, and they should be disinfected regularly especially if they are used as a disposal route.

### Organisms.

Three categories of organism are listed below. Caution must be exercised in the use of these lists, as there is often considerable delay in reports of pathogenicity reaching educational journals. For example, we have found reports of infection associated with Serratia marcescens going back to 1951, but concern was only widely felt in educational circles relatively recently. A more extreme case is that of Pseudomonas aeruginosa, an organism being suggested for school use within the last two years. We have seen reports of pathogenicity in a reference dated 1947 (13) which cites other reviews going back to before 1908. In any case there are

arguments against basing any safety policy on categories or degrees of hazard. Since 'not proven' is the firmest verdict that can ever be given, it is sound judgement to assume guilt.

The Imperial College handbook 'Biohazard' (7) provides a fairly comprehensive list of dangerous pathogens. None of these should ever be used in schools. It is also inadvisable to use any organism or strain which has been reported as causing infection in man and where the hazards are so considerable that they cannot be effectively controlled (5).

List A: Selected micro-organisms drawn from recent science teaching projects which present minimum risk given good practice. It consists of organisms listed in Appendix 2 of D.E.S. Education Pamphlet Number 61 "The use of micro-organisms in schools" (5), reproduced here by kind permission of the Controller HMSO, with additional organisms marked \*, which have been suggested for use in the Scottish C.S.Y.S. Biology course.

### Bacteria

Acetobacter aceti	Rhizobium leguminosarum
Agrobacterium tumefaciens*	Rhodopseudomonas palustris*
Bacillus subtilis	Rhodospirillum rubrum*
Chromatium sp.*	Spirillum serpens
Chromobacterium lividum (a)	Staphylococcus albus
Erwinia carotovora	or
(= E. atroseptica)	Staphylococcus epidermidis
Escherichia coli (b)	Streptococcus lactis
Micrococcus luteus	Streptomyces griseus
(= Sarcina luteus)	Streptomyces scabies*
Photobacterium phosphoreum*	Vibrio natriegens
Pseudomonas fluorescens	(= Benecka natriegens)

### Fungi

Agaricus bisporus	Penicillium chrysogenum
Armillaria mellea*	Penicillium notatum
Aspergillus nidulans	Phycomyces blakesleanus
Aspergillus niger	Physalospora obtusata*
Botrytis cinerea	Phytophthora infestans
Botrytis fabae*	Pythium debaryanum
Chaetomium globosum	Rhizopus sexaulis
Coprinus lagopus	Rhizopus stolonifer
Fusarium solani	Rhizytisma acerinum*
(= Rhizoctonia solani)	Saccharomyces cerevisiae
Fusarium oxysporum*	Saccharomyces ellipsoides
Helminthosporium avenae*	Saprolegnia literalis
Mucor hiemalis	Schizosaccharomyces pombe
Mucor mucedo	Schlerotina fructigena*
Myrothecium verucaria*	Sordaria fimicola

Fungi are seldom directly harmful to man but a number of fungal infections are known and are regularly seen clinically. These involve some species of Mucor, of Aspergillus, Candida albicans ('Thrush') and a number of other fungal pathogens including those causing skin ailments. It is important to handle fungal cultures just as carefully as bacterial ones and to avoid breathing in appreciable quantities of fungal spores. In addition to the slight risk of infections, allergenic reactions are known to occur. Particular care should be taken with cultures of Aspergillus species, since contamination of cultures with pathogenic Aspergilli

is always possible and identification is often difficult.

### Viruses

Bacteriophage (T type, host E. coli)	Potato virus Y* (c)
Cucumber mosaic virus*	Tobacco mosaic virus*
Potato virus X*	Turnip mosaic virus*

### Algae, Protozoa, Lichens, Slime moulds

Though some protozoa are known to be pathogenic the species quoted for experimental work in recent science projects, together with the species of algae, lichens and slime moulds quoted, are acceptable for use in schools.

Notes. (a) This species replaces Chromobacterium violaceum and Serratia marcescens.

(b) Some strains have been associated with health hazards. Reputable school suppliers will ensure that acceptable strains are provided.

(c) Not the virulent strain of virus Y causing veinal necroses as well as the usual symptom of distorted leaf margins. The Department of Agriculture and Fisheries are trying to control the spread of this strain and material infected with it should not be used.

### List B.

The following micro-organisms have been suggested in recent science projects but are, in our opinion, not suitable for use in secondary school courses because of their association with human illness (1, 2, 3, 10, 11, 12, 13, 14, 15).

Chromobacterium violaceum	Pseudomonas aeruginosa
Clostridium perfringens	Serratia marcescens
Proteus	Staphylococcus aureus

### List C.

The following micro-organisms are plant pathogens which have been suggested in recent science projects. They are non-indigenous and should not be used. Indeed they would be difficult, if not impossible to obtain since a licence from the Department of Agriculture and Fisheries is needed before the various culture collections would release them. We are informed that licences for these organisms would not be issued to schools.

#### Bacteria

Pseudomonas solanacearium  
Pseudomonas tabaci  
Xanthomonas phaseoli

#### Fungi

Helminthosporium victoriae  
Uromyces phaseoli

#### Virus

Southern bean mosaic virus

School work can be more than adequately covered using only List A organisms. Teachers wishing to use other organisms, e.g. for sixth year projects, should seek competent advice. SSSERC

is prepared to obtain such advice on behalf of teachers through contacts with MISAC (Microbiology in Schools Advisory Committee), Government Departments and the Dangerous Pathogens Advisory Group. We shall also keep lists A, B and C under review and will issue additions and amendments where necessary.

#### Acknowledgement.

We are grateful to Dr. K.M. Jack H.M.I. (D.E.S. and MISAC) for the help and advice he gave us during the preparation of this article.

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#### Other useful sources.

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## Trade News

The Precision Tool and Instrument Co., makers of spectrometers and other optical instruments have moved to the address given on page 12.

L. Oertling are prepared to service most makes of precision balance on a contract basis. Cost depends on the location and the number of balances to be serviced.

Weldmesh, which was used as the basis of our polythene-coated test-tube and glassware racks (Bulletins 48 and 52) is now subject to a minimum order charge of £50. However, this is reduced to £25 if the material is collected from the B.R.C. depot at Coatbridge. A 25 ft roll of  $\frac{1}{2}$  in, 16 gauge mesh such as we used currently costs £19.75. Regional or district centres may find it worthwhile to buy for their own schools.

Northern Light, suppliers of Cinemoid filters, specialised lamps and lighting equipment are now at the address given on page 12.

Gordon Keeble Laboratory Products, makers of the syringe valve used in a number of our designs, have moved to a new address, given on page 12.

Sensitised Coatings, who will supply chart paper for most makes of recorder more cheaply than the manufacturer, have moved to a new address, given on page 12.

One of the experiments in Section 2 of the new mixed ability integrated science requires a large glass T-piece through which a worm will crawl. For science departments lacking the required glassblowing skill, Philip Harris have produced a 'worm choice tube', B67840/1 at 75p.

The same firm has asked us to point out certain inaccuracies on the flyleaf of their 1977 catalogue. The age range for Scottish Integrated Science is 12 - 14 years, not 12 - 16 as stated, and Heinemann's are not the publishers of the various Scottish syllabuses. These are published by R. Gibson and Sons on behalf of the S.C.E.E.B.

We have had a number of enquiries for spare parts for the Olympus MIC microscope. This is not a standard Griffin and George item, but spares can be ordered through their East Kilbride office. There are no catalogue numbers so that a full description should be given. Typical prices (July, 1977) were eyepiece £1.75; mirror £2.30.

S.S.S.E.R.C., 103 Broughton Street, Edinburgh, EH1 3RZ.  
Tel. 031 556 2184.

(B.R.C.) British Reinforced Concrete Engineering Co. Ltd.,  
Coatbank Street, Coatbridge, Lanarkshire.

The Secretary, Dangerous Pathogens Advisory Group, Room D410,  
Alexander Fleming House, Elephant and Castle,  
London, SE1 6JE.

Gordon Keeble Laboratory Products Ltd., Petersfield House,  
St. Peter's Street, Duxford, Cambridge, CB2 4RP.

Griffin and George Ltd., Braeview Place, Nerston, East Kilbride,  
Glasgow, G74 3XJ.

Philip Harris Ltd., 30 Carron Place, Kelvin Industrial Estate,  
East Kilbride, Glasgow, G75 0TL.

The Secretary, Microbiology in Schools Advisory Committee,  
Polytechnic of Central London, New Cavendish Street,  
London.

Northern Light, West Mill Road, Edinburgh, 13.

L. Oertling Ltd., Cray Valley Works, St. Mary Cray, Orpington,  
Kent, BR5 2HA.

Precision Tool and Instrument Co. Ltd., Coombe Road, Hill Brow,  
Liss, Hants, GU33 7NU.

Sensitised Coatings Ltd., Bergen Way, North Lynn Industrial  
Estate, King's Lynn, Norfolk, PE30 2JL.