

CHEMICAL STERILIZATION

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PHYSICAL conditions such as heat and radiation, and chemical substances which destroy micro-organisms are the two broad groups of agencies used for sterilisation or disinfection. The main fields of application are medicine and public health, the food industries, especially those concerned with highly perishable foods such as milk, and also water.

The classical meanings of the words "disinfection" and "sterility" are still valid, but new terms have been introduced and the classical terms are sometimes used with a modified meaning. Thus the terms "antiseptic", "disinfectant" and "sterilant" are used for similar substances, and "disinfection", "sterilisation", "commercial sterilisation" and "near-sterilisation" for similar processes. Whereas the term "disinfectant" usually implies a powerful germicide with no specific qualifications, "antiseptic" usually implies a germicide with negligible irritant effect on the tissues. The term "sterilant" is used in the food industry, possibly because "disinfectant" so often implies a strong smelling phenolic substance.

Sterility means "incapable of proliferation", and so in the hygienic sense the complete absence of all forms of life. The word is sometimes used more loosely to indicate absence of pathogens, or absence of food spoilage organisms, the small numbers of harmless organisms being ignored. The word sterility should *always* be used in its strict sense; sterility, like virginity, cannot be qualified. A thing is either sterile or not.

In practice the destruction of pathogens or spoilage organisms is often sufficient, and the terms "disinfection", "commercial sterilisation" and "near-sterilisation" are then used. The first is accepted in medical work, and of the other two the latter is to be preferred. Appropriate tests are examination for surviving pathogens, where *E. coli* type 1 is sometimes used as an index which implies dangerous contamination, and for surviving spoilage organisms a maximum total count may be used as a convenient index.

Cleaning and Sterilising—Cleansing

The modern fashion of chemical disinfection has tended to reduce the significance of cleaning, which is of the greatest importance in all aspects of sterilisation. Cleaning may be defined as the complete removal of all extraneous material and especially organic matter, which is food for bacteria. Heat may in time penetrate a film of milk-stone, dried-on-egg, serum or pus, and kill any micro-organisms present, but such films may protect organisms indefinitely against all types of chemical sterilants or radiation. Apart from this, efficient cleaning removes mechanically about 99 per cent of the bacteria on a dirty object, and is, therefore, an integral part of the sterilising process. Cleaning and sterilisation, whether by heat or chemicals, are complementary processes. The term "sanitisation" is commonly used in North America for this combined treatment,

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but this term, although etymologically correct, is not popular in Britain. A convenient term for the combined treatment is "cleansing".

In the food industries cleansing may be defined as "the complete removal of all extraneous matter and reduction of micro-organisms to 1 colony per sq. cm. area or per ml. capacity by standard tests"¹. Organisms surviving are almost invariably spores and resistant cocci of no pathogenic or industrial significance. The validity of this practical definition is well borne out by the fact that when swab and rinse test results are less than 1 per sq. cm. or ml., presumptive coliforms are very rarely, if ever, found. Absence of this most useful index organism usually indicates that all ordinary pathogens and most spoilage organisms have been killed. Spore forming organisms are an unfortunate exception (cf. p. 31 *T*) and when destruction of these is necessary, much more drastic methods must be

TABLE I
SUMMARY OF STANDARD METHODS FOR CLEANING AND STERILISING

	Plant	Tanks and tankers	Pipelines (mains)	Cans	Bottles
Pre-rinsing ..	Cold water	Cold water	Cold water	Cold or warm water	Cold or warm water
Cleaning ..	1. Scrubbing with detergent. 2. Recirculation with detergent 3. Recirculation with $\frac{1}{2}$ -1 per cent nitric acid	Scrubbing with detergent	As for plant	Jetting with or without detergent (brushing)	Jetting or soaking with detergent
Rinsing after detergent ..	Hot water	Hot water	Hot water	Hot water	Warm water
Sterilising ..	Steam, hot water, chlorine, 'quats', nitric acid	Chlorine, 'quats', hot water, steam	As for tanks	Steam ('quats', etc.)	(Hot detergent sterilises) (steam)
Final rinsing ..	Water—if chemicals used	Water—if chemicals used	Water—if chemicals used	—	Water (unless steamed). May be chlorinated 5 p.p.m.

employed. Standard methods for cleaning and sterilising food equipment are given in Table I and precautions to be observed with various materials in Table II.

Bactericides and Bacteriostats

The generally accepted definitions are that a bactericide kills bacteria, whereas a bacteriostat merely prevents growth or proliferation. In practice this distinction may be difficult to sustain: vegetative cells of bacteria which cannot grow usually die, although sometimes very slowly. The difference in effect may be a matter of time, concentration and temperature. Proliferation of cells as measured by colony counts may not be the only form of growth of bacterial cells. All methods of assessing bactericidal and bacteriostatic power are dependent on the technique used. For practical purposes we may assume that if cells cannot be recovered by growth tests after removal of the chemical, then the action is bactericidal. If cells are recovered and grown, then the action is bacteriostatic. It is often difficult to decide when a cell is dead, and this is only one of many

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reasons why laboratory tests may be an unreliable guide to the efficiency of a germicide in practice.

Chemical Sterilisation Compared with Steam and Boiling Water

Where feasible steam or boiling water, or both, are usually the best agents for sterilisation in industry. However, circumstances are often such that chemical sterilisation is preferred. Thus the use of heat may be impossible, as for human skin and tissues, for equipment made of thermolabile material such as glass or plastics, or for heat-sensitive components or finishes. Heat is difficult to apply to large surfaces such as open tanks and walls. It may not be economic if items at various points have to be sterilised rapidly at various times of the day and night. Chemical solutions can easily be stored in concentrated form in any convenient place, and are always ready for use at a moment's notice. An adequate

TABLE II
SPECIAL PRECAUTIONS

	Cleaning	Sterilising
Steel	Any alkali	Not chlorine
Stainless steel	Nitric acid can be used	Chlorine if pH 9 or above.
		Nitric acid possible
Aluminium	No acids or caustic alkalis.	Not chlorine
	At least 25 per cent silicate	
Glass and coatings	Anything except strong alkalis	Anything. Sudden and considerable temperature changes undesirable

supply of steam may not always be available. If equipment is required to be used cold immediately after sterilising, steam and boiling water are obviously unsuitable.

Quite apart from convenience and efficiency, chemical sterilisation is often cheaper than steam. As with detergents, sterilising solutions can be run from equipment requiring the most thorough cleansing to other equipment where the standard required is not so high.

Sterilants Compared with Heat for the Destruction of Spores

Spores constitute a special problem in sterilising work, and unfortunately some spore-formers are extremely important, for example, *Cl. tetani* and *B. anthracis* in medicine, *Cl. botulinum* in canning, and *B. cereus* in pasteurised milk. The only effective ways of destroying spores with certainty are by autoclaving, for example at 120° for 20 minutes; or by dry heat, for example 170° for 2 hours; or by high concentrations of suitable germicides at higher temperatures than those that are adequate for the cleansing of equipment.

The ordinary treatments which are satisfactory for vegetative cells may be useless against spores.

COMMONLY USED STERILANTS IN INDUSTRY

Hypochlorites. Of all sterilants, chlorine or hypochlorite is the best from most points of view. It is cheap, convenient to use, powerful and has a wide antibacterial spectrum. Its greatest disadvantages are

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corrosiveness and odour. The former can be minimised by using in cold solutions and at pH 9 or higher, but it is more bactericidal at neutral or acid reactions. Commercial preparations usually contain 9 to 12 per cent and domestic preparations about 1 per cent of available chlorine. Complete immersion in 150 to 200 p.p.m. hypochlorite will sanitise clean metal and glass in 2 minutes; 10 minutes gives an ample margin for sterility.

Quaternary ammonium compounds. Next to hypochlorite the quaternary ammonium compounds are probably the most useful. They are convenient to use, of very low toxicity to animals, without appreciable odour

TABLE III
STERILISING AGENTS—ADVANTAGES AND DISADVANTAGES

	Steam	Hot water (190° F.)	Chlorine	Quaternary ammonium compounds
Cost	Varies	Varies	Low	High
Convenience	Depends on layout	Recirculatory system necessary	Very convenient	Very convenient
Penetration	Good if adequate supply	Good	Clean plant essential	Clean plant essential
Heating effect	Tanks, etc., may require hours to cool Undesirable stresses may be set up	Less than steam	None or slight	None or slight
Suitability	Very suitable for enclosed systems and small articles in chests	Very suitable for pipelines (mains)	All purposes	All purposes
Persistence	Not persistent	Not persistent	May persist if traces chlorine, etc., remain	
Corrosion	None	None	Extremely corrosive especially at acid and neutral reactions	Not corrosive if thoroughly rinsed away
Odour	None	None	Very marked	None
Toxicity	None	None	Precautions essential	Not toxic in practice

or taste, non-corrosive, and not too expensive. They are very powerful against Gram-positive but are nearly always less effective against Gram-negative organisms, and this is their greatest disadvantage. They are incompatible with soaps and anionic detergents, but can be used with most alkalies and non-ionic detergents. Alkalinity increases their bactericidal power.

They are only very weak detergents. The relative advantages and disadvantages of steam, hot water, chlorine and the quaternary ammonium compounds are given in Table III.

Working concentrations vary from 1 in 1,000 to 1 in 20,000 according to the particular quaternary compound used, the temperature, the time, the alkalinity and the percentage kill desired. Weight for weight they are less effective than chlorine, but their other advantages may outweigh this fact. For detailed information on the quaternary compounds see Glassman², Lawrence³ and Resuggan⁴.

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Alkalis. Although generally regarded only as detergents, caustic alkalis have, in addition, a strong germicidal activity. Thus in bottle-washing, bottles emerging from the detergent section, 3–5 min. in 0.4 per cent NaOH (equivalent) at 145° F., are usually sterile or nearly so⁵.

The bactericidal effect falls rapidly with decreasing pH and is influenced by the nature of the anion (Table IV).

Hot caustic soda solutions at 1–3 per cent are specially useful for killing spores, and this is an advantage claimed for the soaker type of bottle-washing machines.

Iodine. Although very useful as an antiseptic, iodine has certain disadvantages as a sterilant. It has a powerful odour, stains badly, is not very soluble in water, and is expensive. It is readily soluble in iodide solutions and in alcohol, but this method increases its cost, and the solution may be irritant to skin.

Iodophors. Although of recent introduction, it is probable that iodophors will grow in popularity. They are prepared by the action of iodine

TABLE IV
BACTERICIDAL ACTIVITY OF ALKALIS

	pH of 1 per cent solution	Killing time in minutes against <i>B. subtilis</i> spores at 30°
Sodium hydroxide	13.1	Less than 5
Sodium carbonate	11.4	Less than 5
Trisodium phosphate (12 H ₂ O)	12.0	30
Sodium sesquicarbonate	9.9	More than 60

on non-ionic detergents in acid solution, and they have both detergent and sterilising properties. They are in use in North America⁶. It is claimed that the iodine in this solubilised form has about the same killing power as the available chlorine in hypochlorites.

Chloro-compounds. The usefulness of the chlor-phenols and related compounds is well established, but they are odorous and may be irritant. Chloramine-T does not suffer from these disadvantages, but is rather mild in action. Amongst interesting new compounds are dichlorocyanuric acid and dichlorodimethyl hydantoin. It is claimed that these are of extremely low toxicity and are non-irritant.

Dichlorophene is more effective against Gram-positive organisms and fungi than hexachlorophene. Both have recently become popular as antiseptics. The latter has a phenol coefficient of about 40 against *Staph. aureus* and of about 15 against *Salm. typhi*.

Vigorous claims have been made for the efficiency and general suitability of chlorhexidine diacetate and the chloroxynols as antiseptics. The odour of some of these substances makes them unsuitable as sterilants.

Amphoteric or ampholytic compounds. These are mostly alkylated amino acids possessing amphoteric properties, for example dodecyl β-alanine⁷. They possess many advantages according to their sponsors. Thus they are good wetting agents and fairly good emulsifiers. They are

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compatible with anionic, non-ionic and cationic agents. Typical examples are given in Table V.

The Importance of Specificity

Micro-organisms vary widely in their resistance to adverse conditions and to lethal agents. A good example is the difference between *N. gonorrhoea* and the spores of *B. subtilis*. The former quickly dies outside the living animal; the latter may require 1 per cent NaOH for some minutes to achieve a 99.99 per cent kill. Compared with other organisms *Myco. tuberculosis* is highly resistant to most chemicals, especially acids, and yet is rapidly killed by direct sunlight and 70 per cent ethanol. The quaternary ammonium compounds are effective against most Gram-positive bacteria but relatively feeble against the Gram-negative, and

TABLE V
AMPHOTERIC (AMPHOLYTIC) SURFACE-ACTIVE GERMICIDAL AGENTS

Chemical name or formula	Trade name	Manufacturer
Dodecyl-β-alanine	Deriphet	General Mills
Dodecyl-β-aminobutyric acid	Armeen Z	Armour
N-Laurylaminosulphonic acid	Siposan	Sipon
	Miranol	Miranol Chemical
Dodecyl-di(aminoethyl)-glycine	Tego	Th. Goldschmidt

especially *Ps. pyocyanea*. Disinfectants can be highly specific in their action, and in choosing a disinfectant consideration must always be given to this fact.

The Danger of Generalising on the Use of Germicides

Many factors influence the effectiveness of any one germicide for a given purpose. The most important are (i) concentration, (ii) temperature, (iii) time of contact, (iv) pH, (v) amount and kind of organic matter, (vi) types of organism present, and the type selected for assessment purposes, and (vii) physiological condition of organisms. Other factors of minor importance also exert some influence. For this reason it is unsound to make comparisons between disinfectants. Just as it is essential to specify techniques in detail when evaluating disinfectants in the laboratory, so it is essential to specify practical conditions of use when comparing sterilants. The rating of a disinfectant for use as a rinse solution for beer glasses, might prove to be very different when used for pipelines in a dairy. The former must be used quickly in the cold at pH 6-8; the latter can be used for some time at a high temperature at any required pH value.

The Significance of Organic Matter

The commonest cause of failure to achieve sterility, or adequate cleansing, is generally the incomplete removal of organic matter, for

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example milk-stone on pasteurising plant, grease on skin, protein-fat residues on dishes. Such organic matter can interfere in two ways. As a firm film on the object it can act as a barrier between cell and germicide. If it becomes detached and goes into colloidal solution or suspension in the disinfecting liquid it completes with the cells for the disinfectant and so reduces the concentration of disinfectant. Some types, for example the quaternary ammonium compounds, may be inactivated by chemical combination with anionic material, or by adsorption on suspended matter.

The Nature of the Material

Smooth non-absorbent materials such as glass and stainless steel are easy to sterilise. Rough absorbant materials such as all animal and vegetable matter, rubber, plastics and textiles, are difficult if not impossible to sterilise. With these, sterilisation is entirely a matter of contact between sterilant and germ cell. If contact can be established by penetration (for example, of skin by 70 per cent ethanol) or soaking (for example, of blankets and napkins in a quaternary compound or in chlorine solution), then sterilisation or cleansing is quite feasible. Sterilisation may require much longer times, higher concentrations and higher temperatures.

The Nature of the Surface

One of the most important lessons which has been learned by industry and manufacturers of food equipment is the necessity for a smooth finish to, and absence of crevices and dead ends in, all items which come into contact with food, because they are difficult to free from traces of organic matter, and may escape contact with cleaning and sterilising solutions by reason of air-locks. Losses to the food industry from this cause alone must have been great in the past; fortunately the lesson has now been well learned.

Corrosion

The biggest problem in chemical sterilisation, and certainly the greatest disadvantage of chlorine, is corrosion. This may not be realised until it is too late, and damage to the extent of hundreds or thousands of pounds has been done.

Enthusiasm in sterilisation should always be tempered by a fundamental knowledge of the method. As with other developments in industry, manufacturers often acquire experience at the expense of their customers.

The fundamental aspects to consider when using hypochlorite or other corrosive sterilants are (i) to ensure a clean surface, (ii) to use the correct concentration of available chlorine (p.p.m.), (iii) to use cold or barely warm water, (iv) to use for the correct time, for example, 10 minutes, (v) to rinse *immediately* with sterile water (5 p.p.m.), and (vi) to sterilise just before use.

The practice of leaving a closed circuit system full of weak hypochlorite solution overnight is not necessarily wrong, but this must be carefully controlled. The equipment must be corrosion-resistant (for example, stainless steel or glass) and the solution must be weak, alkaline and cold.

Recontamination

Methods of sterilisation are sometimes condemned as the result of tests on the product or on the equipment, when the real cause of failure is recontamination. This is particularly true in bottle washing. Both manufacturers and users of bottle-washing machines are sometimes oblivious of the fact that although their detergent section efficiently cleans and sterilises the bottles (p. 38 *T*), these are then immediately sprayed with a warm rinse having the bacteriological properties of sewage. Although finishing with a mains water rinse it is not surprising that unsatisfactory bottles are obtained. With any machine all rinse tanks should be drained every day and the addition of a sterilant such as hypochlorite or a quaternary compound sufficient to exert a bacteriostatic effect is advantageous. Constant bacteriological control of rinse tanks is essential, and the count should never rise above 1,000 per ml. A final mains rinse should then be sufficient to ensure satisfactory bottles (p. 38 *T*). It is always sound practice to clean immediately after use, and to sterilise immediately before use.

Industrial Practice

The more perishable a food, the better it is as a medium for micro-organisms, and the more important it is to ensure sterility in the food equipment and the containers. Even if the packaged food is sterilised afterwards, a cleansed bottle or can is advantageous. The modern food technologist must *ipso facto* be a microbiologist. Apart from problems of keeping quality, high moisture foods in the pH range 6 to 8, such as milk and meat stews, are good media for the growth of pathogenic organisms, and so there is a double reason for observing the most stringent hygienic precautions in processing.

The Dairy Industry

Milk is our most perishable food and the dairy industry has attracted more legislation than any other branch of the food industry. For many years hot water and steam were the only permitted means of sterilising equipment, and rigorous requirements were laid down in the Milk and Dairies Order, 1926. During the 1939–45 war necessity brought about the introduction of new methods and the Milk and Dairies Regulations, 1949 (now 1959), permitted the use of approved sterilising solutions. The cleaning and sterilising problems in dairying are diverse and (cf. Table I) the spraying of a chemical solution is often more convenient than the use of steam. Dairy plant lends itself particularly to circulation methods of cleansing and in-place methods (see p. 38 *T*) are now being introduced. Cleaning and sterilising in the dairy industry have been revolutionised in the last 30 years and are now highly efficient and scientifically controlled processes. Chemical sterilisation is almost universal in the dairy industry today, but only approved sterilants may be used⁸. Hypochlorites and quaternary ammonium compounds are the commonest but iodophors are being introduced⁹. The ubiquitous use of chlorine, and of acids for descaling and in-place cleaning (p. 38 *T*) has

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been made possible only by the now almost universal adoption of stainless steel for all dairy equipment, apart from glass and plastic for certain types of pipelines. Manufacturers have played an essential part in the revolution in sterilising methods in the dairy industry, and the highest praise must be given to them for their initiative in this respect. In the 1920's equipment was made in tinned copper and iron, and badly designed from the bacteriological aspect. Today a modern processing dairy might be described as a "hygienist's paradise". (For details of sterilising methods in the dairy industry, cf. ref.^{1, 9-12}).

The Brewing Industry

The brewing industry has two important advantages over dairying. Its processes are not controlled by specific legislation, and beer may rightly be regarded as a mild antiseptic, so that the adage "beer is good for you" can be interpreted in more than one sense. Caustic soda and hot water and steam have for long been the classical cleansing agents in the industry. Brewers are extremely jealous of the organoleptic properties of their product and consequently do not countenance the introduction of anything which might affect it. Chlorine is well known to produce highly odiferous chlorphenols with traces of phenolic bodies in foods, and for this reason it is ruled out for certain branches of the food industry. Another aspect is that copper equipment is still common, and so corrosive sterilants are out of the question. Thus the brewing and dairy industries afford an interesting comparison of the influence of materials on sterilising methods in industry. There is also an interesting comparison of the influence of the product on the criteria for disinfection. Pathogens, coliforms and all lactose-fermenters, and *B. cereus* a cause of "sweet curdling" or "bitty cream" are the most feared in milk. With beer, yeasts, lactobacilli and certain streptococci or pediococci are the organisms to be destroyed. Beer has never been the cause of any infectious disease, and so pathogens are of little significance. The quaternary ammonium compounds are suitable for treating beer glasses¹³.

Food Manufacture

Each branch of the food industry has its own problems. As with catering, some of these have blissfully ignored the requirements of hygiene in the past because no problem has been apparent. The Food Hygiene Regulations, 1955, should be regarded as only the start of a drive for hygiene in every branch of the food industry. For some branches of the food industry the requirements are not so severe as those for the dairy industry, so that if methods and standards adopted in the dairy industry are used a satisfactory level of hygiene should be obtained.

The Catering Industry

In the past the level of hygiene in the catering and food industries has often been low. The Food Hygiene Regulations, 1955, constitute a much needed step forward, but there are no instructions regarding sterility or to methods for sterilising food equipment. The difficulties are obvious,

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and there is less need for the introduction of chemical sterilisation than in other industries. A suitable treatment for crockery, cutlery and food utensils is thorough cleaning followed by a short immersion (minimum 30 seconds) in water at about 180° F. Alternatively the use of a germicidal detergent is advisable. For hand washing, where the maximum workable temperature is 120° F., such a preparation can give crockery free from pathogens. Mops and dish cloths should be "boiled" daily.

Bottles

With reasonable care it is easy to cleanse bottles for any industry using mechanical washers. Efficient pre-rinsing and jetting (or soaking and jetting) with detergent solution at the right temperature are more important than the selection of any one detergent. The pharmacist washing bottles by hand may with advantage use a germicidal detergent as advised above for the catering industry. Recontamination (p. 36 *T*) is the greatest danger. Provided that the bottle is efficiently treated, the presence of foreign objects and impervious matter such as concrete or paint does not constitute a public health hazard¹⁴. A most useful and practical book on bottle washing is that by Resuggan¹¹.

In-place Cleaning

With rising labour costs automatic methods of cleaning and sterilising have received a good deal of attention in industry. There is little doubt that in time all cleaning and sterilising will be automatic. The two essentials are a closed circuit system and the complete absence of corrodible items. A typical sequence as used in a modern dairy is as follows.

	<i>Minutes</i>
Cold water rinse	5
Hot detergent circulation	10-20
Cold water rinse	5
Cold chemical sterilant or hot water circulation	15
Sterile water rinse	5

In-place methods are specially suitable for acid methods. In Europe, outside Britain, cold nitric acid 0.5 to 1 per cent is often used to clean and sterilise, and equipment such as pasteurising plant is rarely dismantled. Acid methods are not in general use in Britain (cf. ref.⁹).

Enclosed Spaces

The best way of ensuring a "clean atmosphere" is efficient air conditioning incorporating bacterial filters. Aerosol disinfectants have been accepted as the best "chemical method" of disinfecting air, but there is a natural prejudice against breathing even minute quantities of chemicals. Where a room or chamber can be sealed, formalin vapour is probably still the best, especially for efficiency and cheapness. Ethylene oxide and, in the U.S.A., β -propiolactone, have been used successfully¹⁵. In all vapour methods the relative humidity is an important factor. This should be at least 70 per cent.

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Textiles

The sterilisation of blankets, sheets, pillow-cases, baby napkins and all similar articles is each one a special problem depending on size, thickness, and nature of material. Penetration is essential, and drastic treatment may harm or shrink the fabric. "Boiling", the classical procedure, may be undesirable because of the odours and water vapour produced, and is a cumbersome procedure for large articles. This is a field where chemical sterilisation can be used with advantage. Provided a proper procedure is adopted in hand or machine-washing effective sterilisation can be achieved¹⁶. Quaternary ammonium compounds are commonly used for this purpose, and their ready adsorbability introduces special problems¹⁷. Surface treatment will affect the extent of adsorption. McNeil and others¹⁸ found that vegetative cells of *B. cereus* and *Brevibacterium ammoniagenes* were very sensitive, *Staph. aureus* more variable, and *Proteus* highly resistant to a benzyl quaternary ammonium compound.

Water

The basis of public health is a clean and safe water supply, and the sterilisation of water is the mother of all developments of industrial sterilisation. Water almost inevitably comes into contact with equipment, and mains water is usually of very low bacterial count. However, in factories water can easily become contaminated, and the residual chlorine is usually inadequate to prevent this. The bacteriological quality of all water used in a factory should be carefully controlled¹⁹.

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DISCUSSION

The following points arose out of the DISCUSSION.

One of the difficulties of determining disinfectant activity was the heterogeneous nature of the bacterial population, the interest in which was centred only on those few organisms with high resistance. The use of a single test organism was inappropriate as a measure of the activity of a given disinfectant. A British standard, using staphylococcus as the test organism

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would shortly be published. Phenol might be used to attempt to standardise the organism, using the dilution at which the disinfectant responded to the test organism and applying a fixed correction factor. Phenol was a bad standard for disinfectants with mechanism of action differing from it, but there would be no advantage in relinquishing it as standard unless it was replaced by some other method of quality control. The ability of an organism to grow in the presence of concentrations of phenol at a certain temperature depended on the nutrient concentration. There was an inverse relation between temperature and threshold concentration. Results from a study of the phenomenon of multiplication showed that the materials of the cell exudate contribute to growth. The course of a bactericidal action was not explained by either the mechanistic or the vitalistic theory of disinfection; it appeared that the important factor was the concentration of the active substance on the cell surface, but the ability to saturate a cell surface depended upon the materials present in the environment. Chloroxylenols were still acknowledged for their activity against streptococci, and they had the advantage of retaining their activity on the skin. Their activity against pseudomonads depended on the soap used in their manufacture and likewise the nature of the soap contributed the largest variation in the activity of lysol. For skin disinfectant, alcohol, iodine, the chloramines and possibly one of the quaternary compounds were suitable. The best antiseptic to add to soap was soda; any other addition—apart from some of the mercurials—gave no additional antibacterial properties. For apparatus sterilisation and gaseous disinfection, formaldehyde was still popular, but it had low penetration into woven material. Ethylene oxide was very effective. Beta-propiolactone was also effective but it had been shown to be carcinogenic. When there was any possibility of spore contamination a lysol type of preparation was preferred to a quaternary ammonium compound though bacterial spores had been known to survive in 5 per cent phenol. For the sterilisation of plastic surfaces (provided the surface was in good condition) it was best to clean thoroughly in liquid detergent, rinse, and immerse in 100 ppm available chlorine for one hour. The suitability of the terms “bacteriostat and bacteriostatic” for the 1963 B.P. was queried. Many thought that true bacteriostasis was impossible; a term “bacteriofrene”—a substance which curbed the growth of bacteria—had been proposed.