REVIEW ARTICLE

THE MODE OF ACTION OF ANTISEPTICS

By W. B. Hugo, B.Pharm., Ph.D., F.P.S.

Lecturer in Pharmaceutics, Department of Pharmacy, University of Nottingham, University Park, Nottingham

The study of antibacterial agents falls into two parts, evaluation[1–4] and mode of action. From the latter point of view antibacterial agents may be conveniently divided into three categories, (i) antimetabolites, (ii) antibiotics, (iii) a group of compounds which in this context may be called non-specific and includes such diversified substances as the phenols, phenoxetol, cationic surface-active compounds, certain dyes[5,6], mercuric salts[7], formaldehyde[8], chelating agents[9] and halogens[10,11].

It is proposed to discuss only phenols and their derivatives and cationic surface-active compounds in this review; recent reviews dealing with group (i) are given in references[12–13] and those of group (ii), references[14–18].

Phenols and Their Derivatives

Phenol itself was isolated from coal-tar by Runge and introduced into medicine in 1865 by Lister. Systematic work on its mode of action began in 1872, when it was discovered that phenols were solvents for proteins. Thus Ritthausen[19] and Osborne[20] found that zein dissolved in melted phenol. Kjedahl[21] showed that gliadin was soluble in *p*-cresol, from which it could be precipitated by many organic solvents, while Reichel[22] found that when serum was warmed with anhydrous phenol a clear solution was obtained which underwent no apparent change on boiling. Cooper[23] extended these studies and found that molten phenol dissolved natural and heat coagulated egg albumen. *m*-Cresol was also found to dissolve many proteins.

Meyer[24] showed that the antibacterial action of phenols was parallel to their distribution between protein and water, while Reichel[22] observed that heat coagulated serum and egg white absorbed phenol from aqueous solutions in amounts which were directly proportional to the phenol concentrations, and that the process was reversible. He also showed that *Pseudomonas aeruginosa* absorbed phenol and that the addition of NaCl increased the absorption by both bacilli and heat coagulated serum, and also increased the bactericidal action of phenol.

Cooper[26] concluded that the absorption of phenols by bacteria was the initial stage of their germicidal action. The subsequent action was not the result of a chemical union with the bacterial proteins, but was apparently associated with the de-emulsification of the colloidal system within the cell shown by the precipitation of proteins when a certain phenol concentration was attained. Richardson and Reid[26] showed that the observed action of phenols could be related to the oil: water partition coefficient. The relation of activity to both oil: water and protein: water

distribution coefficients are reconcilable with the findings of Knaysi[27] and other workers that the cell-membrane of some bacteria may be lipoprotein in nature, while lipoid material and protein are both known to be constituents of bacterial protoplasm. Bancroft and Richter[28] reviewing the chemistry of disinfection concluded that germicidal action is similar to narcosis in higher organisms and concluded also that phenol acts by directly coagulating cell colloids. They were able to observe directly the coagulation of the cell proteins of *Bacillus megaterium* and *Aerobacter aerogenes* by phenol.

Fogg and Lodge[29] summarising a series of studies on the effects of sublethal concentrations of various antibacterial agents on *A. aerogenes*, confirmed that the antibacterial activity of phenols could be related to the distribution coefficient between an aqueous system and olive oil. Those phenols in which the ratio of the concentration in oil to the concentration in the aqueous system was high had the greater antibacterial activity. The criteria of activity were the effect on cell division, growth rate and on the lag phase of growth. Because these factors were affected to the same extent it was concluded that there was a general mechanism by which phenols killed bacteria, namely the coagulation of cellular protein. Phenols were also found to differ from substances such as sulphanilamide, acriflavine or methylene blue in that it was not possible to train or adapt the test organism to grow in inhibitory concentrations of the phenols. This was taken as further evidence that phenols exert their antibacterial effect by a non-specific mechanism. Labes[30] favoured a chemical rather than a physical mechanism and suggested that phenols exerted their action by combining with hydroxy groups in the cell protein. This may well be one of the mechanisms by which protein coagulation or precipitation is effected.

Pulvertaft and Lumb[31] found that antiseptics at bacteriostatic concentrations caused the lysis of certain bacterial cultures. The organisms tested varied in the extent to which lysis occurred, advanced lysis being found with staphylococci, pneumococci, *Bacillus subtilis* and several strains of *Escherichia coli*; less-marked lysis was found with *Shigella dysenteriae*, very little lysis was found with *Streptococcus haemolyticus* and a non-haemolytic streptococcus. With some organisms although lysis was encountered at a low concentration of the antiseptic it did not occur at the higher concentration. For example, at a phenol concentration of 0·045 per cent a culture of *E. coli* underwent complete lysis, whereas at 0·54 per cent no lysis occurred. A suggested explanation of these facts was that at the lower concentration lytic enzymes present in the cell were activated by phenol. At the higher concentration, the lytic enzymes were themselves inhibited and thus no lysis occurred. The lytic phenomenon was not seen in older cultures.

Hotchkiss[32] stated that when bacterial cells were treated with tyrocidine or certain detergents a leakage of phosphorus- and nitrogen-containing substances occurred. Gale and Taylor[33] studied the kinetics of the leakage of individual amino acids from the internal environment of *Streptococcus faecalis*. Amino acid release was estimated by measuring the

carbon dioxide produced in the presence of the specific amino acid decarboxylase. Tyrocidine, Aerosol OT, cetrimide and phenol were examined. Phenol, in a final concentration of 10 mg./ml. (1 per cent) caused in 40 minutes a leak of glutamic acid almost as great as that released by boiling the cells. A concentration of 0·33 per cent phenol caused a leakage of about one quarter of the amount released by 1 per cent phenol. A concentration of 2 per cent phenol also caused a leak, but the glutamic acid decarboxylase was itself inhibited at this concentration of phenol. The leakage of lysine could not be followed satisfactorily as lysine decarboxylase was sensitive to the phenol concentrations used, although an evolution of carbon dioxide was observed before the decarboxylase was inactivated. Previous experiments had determined that a concentration of 2 per cent of phenol sterilised a culture containing 10^8 cells/ml. in 30 minutes and a concentration 0·25 per cent prevented the growth of an innoculum of 10^6 cells/ml. after 48 hours incubation at 37°. It was concluded from these experiments that phenol owes its disinfectant action to an effect on the bacterial cell wall whereby essential constituents of the internal environment are released.

Deere[34] in his studies on the phenomenon of lactose fermentation in *E. coli. mutabile* noticed that those which did not ferment lactose could be made to do so by exposure to thymol or toluene, or by drying. These treatments were thought to increase the permeability of the cells to the substrate, since they were not deficient in the "lactose" enzyme. Maurice[35] studied the effect of phenol on the permeability of *E. coli* to basic dyes. He found that as the dye was absorbed, the optical density of the suspension increased and by following optical density changes he was able to follow the course of absorption. He found that phenol increased the rate of penetration of the dyes into the bacterial cell, and also that calcium ions were able to inhibit this effect.

More recently Haydon[36] observed the effect of phenol on the electrophoretic mobility of *E. coli* and concluded, as similarly did Gale and Taylor[33], that phenol caused leakage of metabolites from the cell; also lysis and death were related. Whether lysis caused death or death resulted in lysis was not known. Lysis and leakage similar to that produced by detergents or phenol are known to be produced by heating the cells, thus lysis is a result of death by heat in this instance.

Quastel and Whetham[37] studied the ability of the monohydric alcohols (C_1 to C_8) to function as substrates for *E. coli.* It was found that methyl alcohol and the alcohols from butyl, C_4, to octyl, C_8, were unable to function as substrates and furthermore they inhibited or retarded the dehydrogenation of other substrates. Ethyl and propyl alcohols exhibited a dual role acting as substrate at low concentrations and at higher concentrations as inhibitors of the dehydrogenation of other substrates. The observed inhibitory effect was thought to be due to adsorption at the surface of the organism. It was noted also that the amount of inhibition increased with the chain length of the alcohol. The inhibitory action of phenol and a number of organic solvents on dehydrogenases was also investigated. The method was to place the substrate

in phosphate buffer, bacterial suspension, methylene blue and inhibitor in a Thunberg tube and then to measure the time to reduce the dye. A comparison of these reduction-times gave a measure of the relative inhibitory power of the substances tested. *cyclo*Hexanol and *cyclo*hexene were found to be much more toxic to succinic dehydrogenase than phenol. Both viability and the metabolic activity of the cells should be investigated since Cathcart and Hahn[38] have shown that acetone-killed bacterial cells reduce methylene blue.

The mechanism of action of inhibitors was extended by Quastel and Wooldridge[39]. They treated the washed suspensions of *E. coli* in phosphate buffer with the inhibitor for a given period. The cells were then washed free from inhibitor and resuspended in phosphate buffer, and their dehydrogenase activity measured. The results thus indicate irreversible decreases in dehydrogenase activity brought about by the various inhibitors. They found that glucose, glycerol and mannitol dehydrogenase were susceptible to all the inhibitors examined; lactate and formate dehydrogenase were more resistant (Table I).

TABLE I

THE REDUCTION TIMES IN MINUTES FOR METHYLENE BLUE AFTER EXPOSURE TO VARIOUS INHIBITORS

Substrate	Inhibitor and exposure time				Control untreated
	Phenol 1 per cent 5 mins*	Propanol 30 mins	Toluene 5 mins	*cyclo*Hexanol 5 mins	
Succinate	44·0	∞	21·5	∞	14·2
Lactate	7·5	2·5	8·7	∞	7·7
Formate	4·2	25·7	4·7	49	4·0
Glucose	∞	∞	∞	∞	11·2
Glycerol	∞	∞	∞	—	5·2
Mannitol	∞	∞	∞	∞	7·2

* Subculture showed a few discrete colonies.

Quastel and Wooldridge[39,40] believed that the site of dehydrogenation reactions was at the cell surface and that the reason for the selective action of toluene in inhibiting the dehydrogenations of the sugars was that the responsible enzymes were associated with lipoid material or were lipoidal in nature; although it was also considered that the permeability of the cell might be affected, the role of an altered permeability was not considered to be significant. Cook[41] later showed that the velocity of acetate oxidation by *E. coli*, measured in the Barcroft respirometer, was slightly faster in the toluene-treated cell than in the untreated cell and suggested that this effect was due to an increase in cell permeability. He was also able to show that toluene-treated cells still retained their ability to oxidise lactate, formate and succinate when molecular oxygen as well as methylene blue were the final hydrogen acceptors. Formate was oxidised to completion and succinate was converted to fumarate in 82 per cent yield and lactate to pyruvate in 76 per cent yield.

No further work on the action of antiseptics on enzymes appeared until 1937, when Bach and Lambert[42,43] studied the effect of antiseptics and solvents on certain dehydrogenases of *Staphylococcus aureus*. Their method was to expose the washed cells to the antiseptic for 30 minutes

at 40° both in the presence and absence of the substrate. Toluene, benzene and *cyclo*hexanol were used in saturated aqueous solutions. The cells were then washed three times with water and their dehydrogenase activity measured by the Thunberg method. With the lactic dehydrogenase[42] it was found that toluene, benzene, *cyclo*hexanol, acetone and phenol never produced total destruction of this enzyme; also the presence of lactate reduced the apparent inhibition of the dehydrogenase. In contrast iodine 1×10^{-4}, mercuric cyanide 1×10^{-6}, and copper sulphate 1×10^{-2} completely destroyed the enzyme and no protection was afforded by the substrate.

The work was extended[43] to the glucose, succinic, formate, butanol, pyruvic, fumaric and glutamic dehydrogenases. The glucose, formate and butanol systems, like the lactate were only partially inactivated by benzene, toluene, acetone or phenol treatment, while systems activating succinate, fumarate, pyruvate and glutamate were completely destroyed. Results for benzene and phenol are given in Table II. Except for the system glucose: acetone, the presence of the substrate exerted a protective effect. Iodine, mercuric cyanide and copper sulphate completely inactivated all these enzymes whether substrate was present or not.

TABLE II

THE EFFECT OF INHIBITORS ON CERTAIN DEHYDROGENASES OF *Staph. aureus*

Inhibitor	Suspension	Substrate Reducing time, minutes							
		Lactate	Glucose	Formate	Butanol	Succinate	Fumarate	Pyruvate	Glutamate
Phenol 0·1 per cent	A	2·5	7	7·25	9	40	26	33	29
	B	4	11	17·5	12·5	∞	∞	∞	∞
	C	2·3	6	8·75	9·75	∞	65	∞	20
Benzene saturated aqueous solution	A	3	6·5	28	10	52	25	17	30
	B	3	7	52	12	>152	127	19	125
	C	3	3	13	20	—	—	—	—

Suspensions. A. Untreated. B. Treated with inhibitor for 30 minutes at 40°. C. As B but substrate present.

Sykes[44] also investigated the action of substances used in medicine as antiseptics on the succinic dehydrogenase of *E. coli*. His method differed from that of Quastel and Wooldridge, and Bach and Lambert in that the bacteria were not washed after treatment with the antiseptic. The cells were treated with the antiseptic at room temperature and after an interval, substrate, buffer and methylene blue were added. The tubes were then evacuated and filled with nitrogen, placed in a water bath at 37° and the time at which 90 per cent reduction of the dye was seen, compared with a similar tube containing only 10 per cent of the methylene blue. For phenol, viable counts were made on the suspensions after treatment; with other antiseptics subcultures were made to test for residual viability. Sykes concluded that the concentration of *p*-chlor-*m*-cresol, hexylresorcinol, *p*-butylphenol, amyl-*m*-cresol, phenol and ethyl, *iso*propyl, *n*-butyl

and *n*-amyl alcohols required to completely inhibit the succinic dehydrogenase of *E. coli* was always slightly in excess of the minimum lethal concentrations.

Dagley and colleagues[45] grew cells of *A. aerogenes* in a synthetic medium containing glucose, potassium dihydrogen phosphate, ammonium sulphate, and magnesium sulphate, and found that a progressively increasing lag-phase was introduced by increasing the doses of phenol. This lag could be abolished or reduced by the addition of a culture filtrate of the synthetic medium in which the organisms had been growing, or by L-leucine, DL-methionine, or L-glutamic acid and also by α-ketoglutaric or succinic acids. On the other hand other amino and carboxylic acids were found to increase the lag period, DL-aspartic or fumaric acids being examples. The bacteriostatic effect of phenol was thought to be due to its inhibition of the synthesis of metabolites essential for rapid cell division. Roberts and Rahn[46] selected one substrate, acetate, and investigated the action of germicides on its oxidation and dehydrogenation by *E. coli.* As catalase was thought to be implicated in using acetate, the effect of the germicides on this enzyme was also investigated (Table III).

The effects of sublethal concentrations of phenol were found to be irreversible by dilution with water. The general effect emerges from a

TABLE III

THE EFFECT OF PHENOL ON CERTAIN METABOLIC ACTIVITIES OF *E. coli*

Enzyme system	Per cent inhibitions in phenol concentrations of		
	0·075 per cent	0·15 per cent	1·2 per cent
Acetate oxidation	20	48	96
Acetate dehydrogenation	0	0	96
Catalase activity	9	10	38
Effect on growth	retarded	inhibited	lethal

consideration of the data with phenol that enzyme systems differ in their sensitivity to phenol and that even at lethal concentrations some oxidase and dehydrogenase activity persists. The catalase activity is apparently inhibited only to about 38 per cent.

The action of 2:4-dinitrophenol (DNP) and certain other nitrated and halogenated phenols on the metabolic reactions of microorganisms are of considerable interest. Shoup and Kimler[47] found that DNP at first stimulated and then depressed the rate of respiration of certain luminous bacteria. Stimulation was not however found with all substrates tested; thus Krahl and Clowes[48] and Genevois and Creach[49] detected no stimulation in the respirator of yeast with lactate, pyruvate or glycerol as substrates.

Attempts to explain the mechanism of the stimulatory action of DNP have been made by several workers. Stenlid[50] working with young wheat roots believed that dinitrophenols render the substrates more accessible to the enzymes by breaking down cellular compartmentation, while

Vandendriessche[51] thought that the stimulation was due to a specific stimulation of phosphohexokinase. Loomis and Lipmann[52] made the fundamental discovery that in a cell-free homogenate from rabbit kidney oxidising glutamic acid, *m*-dinitrophenol prevented the phosphorylation while oxidation was unaffected or even slightly stimulated. These workers believed that the general mechanism of dinitrophenol stimulation was due to the "uncoupling" of a phosphorylation and oxidation in a known biochemical reaction, and the term "uncoupling reagent" was coined for compounds which exhibited this property.

It is well known that washed suspensions of bacteria do not always oxidise a substrate to completion. With *E. coli* and *Bacterium alcaligenes*, Cook and Stephenson[53] showed that although formate was oxidised to completion, the following substrates were oxidised only to the extent shown: lactate 66, glucose 66, pyruvate 60 and acetate 75 per cent. Barker[54] found incomplete oxidation of certain substrates by suspensions of the alga *Prototheca zopfii*, the figures being glycerol 29, glucose 30, ethanol 46 and acetate 50 per cent. Giesberger[55] found a similar situation with different species of spirillum and made the suggestion that the unmetabolised fraction of the substrate might be assimilated. Clifton[56] confirmed the incomplete oxidation of substrate by *E. coli* and extended it to *Pseudomonas calco-acetica*. He found, however, that in the presence of sodium azide (NaN_3) or DNP, oxidation proceeded to completion, the inference being that these reagents prevented assimilation and the substrate was wholly oxidised. A more detailed study of the phenomenon was made by Clifton and Logan[57] who confirmed that washed suspensions of *E. coli* do not oxidise acetate, lactate, glycerol, fumarate, succinate and glucose to completion. The oxidation of glucose, pyruvate, acetate and glycerol proceeded to completion in the presence of DNP or NaN_3. The oxidation of fumarate and succinate was inhibited at very low concentrations of the reagents. Later experiments attempted to prove that assimilation had in fact occurred, and Siegel and Clifton[58] demonstrated an increase in cell weight, but this did not account for the discrepancy between the calculated and measured increase in cell weight for the substrates investigated, but the degree to which a 100 per cent carbon recovery may be demonstrated is obviously dependent on the accuracy of measuring all the metabolic products.

Other workers have shown that DNP and NaN_3 are not universal uncoupling reagents. Burris and Wilson[59] using washed suspensions of the root nodule bacterium *Rhizobium trifolii* noticed that in the presence of DNP, although increased O_2 consumption of the suspensions with glucose did occur, oxidation did not proceed to completion. Further, when added after all the glucose had disappeared from the external medium, DNP caused a further consumption of oxygen which suggested that one of the actions of DNP was to cause intracellular material to be oxidised.

Pickett and Clifton[60] observed that the assimilation of glucose by yeast cells under aerobic conditions, although inhibited by DNP, was not accompanied by an increase in oxygen consumption. Hotchkiss[61] using washed suspensions of staphylococcus cells noticed that DNP stimulated

oxygen consumption and decreased the assimilation of inorganic phosphate. Gale[62] found that the assimilation of free glutamic acid by *Staph. aureus* required energy which could be made available by the co-fermentation of glucose. DNP inhibited the assimilation of the amino acid, but this was not due to inhibition of glucose fermentation. With *Str. faecalis* an increase in the internal concentration of glutamic acid was observed. This was shown to be due to the interference within the cell of the normal glutamic acid metabolising system by the DNP. Rothstein and Burke[63] studied the effect of DNP on the endogenous CO_2 production of "starved" yeast cells and found that in the absence of the phenol no measurable quantities of CO_2 were produced; in the presence of phenol, however, an appreciable production of CO_2 was induced; the source of the CO_2 and ethanol produced, was intracellularly stored glycogen, and the main action of the DNP was to cause this reserve material to be fermented.

Simon[64,65] made a detailed study of the action of phenol and certain nitrated phenols on the respiration, assimilation and fermentation of glucose by a yeast isolated from a sample of commercial baker's yeast. 3:5-Dinitro-*o*-cresol in concentrations of 10^{-5}M stimulated the amount of oxygen used by as much as 1·7 times. At high concentrations the consumption of oxygen was inhibited and aerobic fermentation appeared, which reached a peak at about 10^{-4}M and then was progressively inhibited. In the absence of inhibitors the use of oxygen by washed yeast suspensions with glucose is about only half that required for complete oxidation according to the equation $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$. But in the presence of 2×10^{-5}M of the cresol, an oxygen consumption corresponding to 93 per cent oxidation was obtained. It was assumed that the action of the cresol at concentrations rising to 10^{-5}M was to prevent assimilation of glucose, and this accounted for the increased use of oxygen, that proportion of glucose assimilated by normal cells being now oxidised by molecular oxygen. *o* and *p*-Nitrophenol gave similar results. Phenol, although it did not stimulate respiration, did inhibit assimilation.

Simon[66] believed from an analysis of his result that dinitrocresol acts as an uncoupling agent and that both oxidative assimilation and the rate of glycolysis are controlled by the level of energy-rich phosphate. He concluded that there was little doubt that both the stimulation of respiration and the inhibition of oxidative phosphorylation is profoundly influenced by nitrophenols. The results obtained with phenol provided no clear evidence of any effect on phosphorylation.

The general effect of nitration or chlorination of phenol is to increase its bactericidal properties but the additional uncoupling effect on metabolism should not be overlooked. Higher concentrations are, of course, necessary to kill bacteria than those which cause the uncoupling effects. Suter[67] gives extensive data relating structure and bactericidal properties of phenols and substituted phenols.

Hugo[68,69] studied the effect of phenol and phenoxetol on the oxidation of certain substrates by washed suspensions of *E. coli*. It was shown that

phenol and phenoxetol 0·1 to 0·2 per cent caused a stimulation (10 to 20 per cent) of the rate of oxygen consumption when glucose, mannitol and lactose were used as substrate; these same concentrations caused a marked inhibition (10 to 15 per cent) of the rate of oxygen consumption when lactate, pyruvate, acetate or succinate were the substrates. No changes in the viable population and no uncoupling effect could be demonstrated. It was thought that the enzymes mediating the stimulated reaction were situated within the cell and the first action of phenol and phenoxetol was to increase the permeability thus facilitating the access of substrate to enzymes within the cell. The marked inhibition of lactate and succinate activity could be interpreted if the enzymes responsible for their oxidation were located at the surface of the cell. Partial confirmation was obtained[70] by comparing the action of these two antiseptics on a disrupted preparation of *E. coli* which was capable of oxidising glucose and lactate. No stimulation of glucose oxidation was obtained with the disrupted preparation and the oxidation of lactate appeared less sensitive in the disrupted preparation than in the intact cell. Disruption of the cell would have the effect of destroying the status quo of enzyme location, thus the diffusion barrier represented by a cell wall or cell membrane would no longer function, and a reaction stimulated by an increase in the permeability of the barrier would not be expected to undergo stimulation in a disrupted preparation. Similarly, enzymes located at a cell surface and therefore immediately exposed to the action of an adverse environment might appear less susceptible when the cells have been disrupted. A review dealing with the special problems of enzyme location in microbial cells was made by Alexander[71], and of enzyme isolation by Hugo[93].

CATIONIC SURFACE-ACTIVE COMPOUNDS

Miller, Baker and Harrison[72] showed that a very low concentration of an alkyldimethylbenzylammonium chloride inhibited the respiration and glycolysis of pure cultures of organisms found in the early lesions of human teeth. They used washed suspensions of these organisms and measured the respiration in the Warburg apparatus. They extended their work[73,74] to a systematic study of the effects of anionic, cationic and non-ionised synthetic detergents on the aerobic and anaerobic respiration of glucose by washed suspensions of *Staph. aureus*, *Staph. albus*, *Micrococcus tetragenus*, *E. coli*, *Proteus vulgaris*, *Salm. paratyphi*, *Sarcina lutea*, *Pseudomonas aeruginosa*, *A. aerogenes*, *Shig. dysenteriae* and a lactobacillus and concluded that all the cationic detergents were effective inhibitors of respiration at concentrations of 1 : 3000 and that the Gram-negative and Gram-positive organisms were equally affected. They also noted that some of these detergents stimulated bacterial metabolism at subinhibitory concentrations, a phenomenon they found much more commonly amongst the anionic detergents. Later these workers showed that depression of metabolism was roughly parallel with killing. The test of time to kill was based on an end point method capable only of detecting all alive, or all dead.

Kuhn and Bielig[75] suggested that quaternary ammonium compounds

could react with protein causing in this way the death of the cell, possibly by a disorganisation of the cell membrane. Following up Kuhn and Bieligs' suggestion, Hotchkiss[32] was able to show the leakage of nitrogen- and phosphorus-containing compounds from staphylococci when treated with a variety of surface-active compounds including dodecylamine, and alkyldimethylbenzylammonium chloride and the polypeptide antibiotic tyrocidine. Gale and Taylor[33] made a detailed study of the action of tyrocidine and an anionic (Aerosol O.T.) and a cationic (cetrimide) detergent on the leakage of free amino acids from the internal environment of *Str. faecalis*. They prepared cell-free amino acid decarboxylases of high specificity which enabled individual amino acids to be estimated[76]. Gale and Taylor concluded that the lytic action of tyrocidine, cetrimide, Aerosol O.T. and phenol was sufficient to explain the disinfecting action of these substances. Similar results were obtained with *Staph. aureus* and *Saccharomyces cerevisiae*.

Salton[77] made a detailed study of the "leakage" phenomenon using cetrimide and six different organisms. He measured quantitatively the leak from the cell of material absorbing in the ultra-violet region of the spectrum at 260 mμ, inorganic phosphorus, total phosphorus, pentose, glutamic acid, purines, pyrimidines and their derivatives. This work revealed a simple relation to exist between the leak of 260 mμ absorbing material, glutamic acid and inorganic phosphorus from *Staph. aureus* and *Bacillus pumilus*. A similar relation was found for *E. coli* except that glutamic acid was not released from this organism. Treatment of suspensions with sufficient cetrimide to sterilise them released amounts of cell constituents comparable to those released by placing the cells in boiling water. When smaller amounts of cetrimide were used, a quantitative relation was found to exist between the amount of detergent present, the proportion of cells killed and the amount of 260 mμ absorbing material released. The form of the curve relating the uptake of this detergent from solutions containing it in varying amounts was shown in the case of *Staph. aureus* and *E. coli* to be that of a typical adsorption isotherm, and the maximum amounts of cetrimide adsorbed varied for each of six bacterial species tested.

Salton[78] also grew *Staph. aureus* and *Pseudomonas fluorescens* on a medium containing ^{32}P. An increased leak of ^{32}P was demonstrated when the cells were treated with cetrimide. Further evidence for the cytolytic damage was obtained by Salton, Horne and Cosslett[79], who studied electron micrographs of *Staph. aureus, Str. faecalis* and *E. coli* treated with cetrimide, and found that using concentrations of 90 μg./ml. the cytoplasm shrunk away from the cell wall. With concentrations of 900 μg./ml. the cell wall was stripped off. Meisel and Umanskaya[80] had observed, with the ordinary optical microscope, that the protoplasm of yeast cells shrunk away from the cell wall when treated with cetylbenzalkonium chloride.

The change in electrophoretic mobility of bacterial cells has been used to investigate the bacterial surface and the effect of substances on the surface charge[81]. Dyar and Ordal[82], studied the electrophoretic mobility

of ten microorganisms and the effect of 2-methyl-7-ethylundecanol-4-sulphate (STS) and cetylpyridinium bromide (CPC) on mobility. They found that with CPC the following general picture was obtained with all their bacterial species, viz., decrease, reversal and stabilisation of the surface charge, although the degree of the change and the concentration of CPC to produce it varied with the species of bacteria tested. In contrast, when anionic detergents were used, considerable differences were seen in the responses of the bacterial species. For example, using *Ps. aeruginosa* the mobility showed a small but definite increase, while using two strains of *E. coli* no change in mobility was recorded between 0 and 10^{-3}M STS. With *Spirillum volutans* at a concentration of STC of 10^{-5}M the mobility showed a marked increase.

McQuillen[83] reinvestigated the effect of cetrimide on the electrophoretic mobility of *Staph. aureus*, *E. coli* and *Str. faecalis*. McQuillen's findings agreed with those of Dyar and Ordal for the Gram-negative *E. coli*; that is, the mobility of the organism was decreased, became zero and finally its direction was reversed with increasing concentrations of cetrimide. The behaviour of the Gram-posititive organism studied by McQuillen differed from that found by Dyar and Ordal[82], who had reported that the Gram-positive *Staph. aureus* showed the same type of behaviour as the Gram-negative *E. coli.* McQuillen found that with Gram-positive organisms after a small initial decrease in mobility there was an abrupt rise above 50 μM to a maximum at 100 μM followed then by a decrease in mobility so that these organisms bore only a small residual positive or negative charge in the presence of 250 μM cetrimide. This maximum at 250 μM is seen when the cell surface is saturated with the detergent, measured by the adsorption techniques. McQuillen concluded that for the Gram-negative organism the increasing quantities of the detergent cation combine with the negatively charged organism resulting in the reduction and eventual reversal of the net negative charge. For Gram-positive organisms this explanation was not adequate. As stated above, it had been found that the maximum of the mobility concentration curve coincided with saturation of the cells with the detergent. To attempt to explain this mobility maximum several hypotheses were suggested. A removal of an external layer from the cell may expose a layer with a greater inherent negative charge; or a general reorientation of the surface layers of the cell may in some way reveal a greater number of more negatively charged groups. Thirdly, material leaking from the cell may be reabsorbed on to the surface of the cell and thus again increase the apparent net negative charge. McQuillen felt it was not possible from the evidence obtained to suggest any particular site on the cell surface at which cetrimide might appear to be specifically absorbed.

Further evidence for the occurrence of leakage from bacterial cells treated with a surface-active cationic germicide was obtained by Eggenberger[84]. It was found that on the addition of dodecylammonium chloride to thrice washed *Staph. aureus* cells suspended in conductivity water, an increase in the equivalent conductivity of the system was noted, presumably due to the leak of electrolytes from the cells. When autoclaved

bacteria replaced the living suspension, the increase conductivity on exposure to the surface-active agents could not be detected, suggesting the heat treatment also caused a leak of electrolyte and further leakage could not be induced by the detergent. This was later confirmed by Salton[77] who demonstrated a leak of 260 mμ absorbing-material from bacteria that had been kept at 100° for 10 minutes. Thus it was concluded that the leak of electrolytes from bacterial cells could be effected by heat treatment as well as by treatment by surface-active agents. The data suggested to Eggenberger and colleagues that the released material could not have been entirely inorganic ions but, because of the increases in equivalent conductivity obtained, must have been due to some other material.

Ordal and Borg[85] studied the effect of cetylpyridinium chloride and sodium dioctylsulphosuccinate on the oxidation of lactate by *E. coli* and *Staph. aureus* using both molecular oxygen and methylene blue as hydrogen acceptors. These workers found that the lactate methylene blue system of *Staph. aureus* was far more susceptible to the action of both agents than was the same system in *E. coli.* When molecular oxygen was the final hydrogen acceptor, lactate oxidation by *Staph. aureus* was inhibited by both compounds in contrast to *E. coli* to which only the cationic (pyridinium) compound was inhibitory. It was concluded from this work that the terminal oxidation enzymes of lactate oxidation of *E. coli* are more susceptible than those responsible for the reaction in which an artificial carrier was the final hydrogen acceptor, and it was suggested that the cytochrome system responsible for the mediation of the reduction of molecular oxygen with *E. coli* was either more susceptible or more accessible to the action of the surface-active agent.

Sevag and Ross[86] made a systematic study of the action of the cationic detergent, benzalkonium, on certain enzyme systems of bakers' yeast. It was found that at concentration of 0·1 per cent the 545 to 565 and 605 to 625 mμ absorption bands of cytochrome c in yeast cells were reversibly reduced in intensity. Quantitative measurements of the reduction in intensity of the bands were not made, their value being estimated by inspection in a hand spectroscope. The cytochrome-cytochrome oxidase system was investigated by following the oxidation of *p*-phenylenediamine colorimetrically and manometrically. Inhibition of this system was found to be complete at a benzalkonium concentration of 1 : 35,000 and a yeast-benzalkonium ratio between 10 : 1 and 20 : 1. The oxidation of glucose by molecular oxygen was inhibited to the extent of 91 per cent at yeast-benzalkonium ratios of 30 : 1. When methylene blue replaced oxygen as the final acceptor, inhibition was 97 per cent for yeast-benzalkonium ratios of 30 : 1 to 40 : 1. Further experiments attempted to relate the inhibition of growth with inhibition of respiration of glucose in phosphate buffer. At benzalkonium concentrations of 1 : 55,000 to 1 : 220,000 a stimulation of oxygen consumption of 54 to 60 per cent was obtained, the inhibition of growth was stated to be from 34 to 83 per cent.

Roberts and Rahn[87] measured the effect of disinfectants on the dehydrogenation and oxidation of acetate and the catalase activity in *E. coli.* Enzymic activities in the three reactions were followed by measuring the

rate of decolorisation of methylene blue with the Thunberg method, the rate of oxygen consumption in the Warburg respirometer and the amount of undecomposed hydrogen peroxide remaining in the reaction system. The authors were seeking to test whether the killing of bacteria was due to the inhibition of energy-furnishing enzymes and concluded that with phenol and the two cationic compounds, retardation of growth occurred at a concentration which had little effect on energy production measured by these three biochemical reactions. At bacteriostatic concentrations the amount of enzyme inactivation varied, the acetate oxidation system appearing to be the most susceptible. At lethal concentrations, acetate oxidation and dehydrogenation was retarded to the extent of 96 per cent, but catalase activity was again less susceptible.

Krebs[88], in a general study of the decarboxylation of glutamic acid and its monoamide, glutamine, by washed suspensions of *Clostridium welchii* noted that cetrimide accelerated the decarboxylation of these two compounds as measured manometrically by the rate of CO_2 evolution, while an anionic detergent, sodium dodecylsulphate, even at 0·05 per cent concentration, inhibited this decarboxylase. The histidine decarboxylase on the other hand was inhibited by cetrimide, but not affected by sodium dodecylsulphate. Krebs suggested that one explanation of the acceleration was that the permeability of the bacterial cells to the substrate was increased. To test this hypothesis Hughes[89] measured the effect of cetrimide on the rate of decarboxylation of glutamate and glutamine using cell-free preparations of *Cl. welchii, Proteus morganii, E. coli* and *Str. faecalis.* Contrary to the expectation, cetrimide accelerated the decarboxylation of glutamine and glutamic acids by *Cl. welchii, P. morganii* and *E. coli* to the same extent whether intact cells or cell-free extracts were used, and Hughes concluded that the main action could not be due to the effect on permeability. At low substrate concentrations, however, the degree of acceleration with intact cells was slightly higher than with extracts, and it was suggested that under these conditions the acceleration might be due to an increase of cell permeability. An examination of the remaining experimental data suggested that cetrimide increased the apparent affinity of the enzyme for its substrate. This in turn could be accounted for by (i) increased local concentration of substrate in the vicinity of the enzyme, (ii) alteration in the properties of the enzyme by reacting with an added substrate, or (iii) removal of an enzyme inhibitor. Of these hypotheses the third was thought to be the only one which explained the stimulation of decarboxylase and glutaminase activity. The possible nature of the inhibitor was not discussed. No stimulation was observed with decarboxylases for other amino acids when five bacterial and two plant sources of these enzymes were tested.

Knox and colleagues[90] studied the effect of five quaternary ammonium and pyridinium detergents on certain metabolic reactions of *E. coli.* These were, lactate oxidation and dehydrogenation, glucose and hexose diphosphate oxidation and glycolysis, the oxidation of pyruvate, formate, alanine and succinate, arginine decarboxylation and finally aldolase activity. In the experiments on the inhibition of glucose oxidation the

kill per cent was estimated at the end of the experiment by means of a viable count on the contents of the Warburg flask. In certain instances cell-free enzymes were prepared and the effect of the detergents on the activities of the cell-free enzymes compared with the effect on the intact cells. The concentration of each of the five detergents expressed as μg./mg. of bacterial nitrogen to produce 50 per cent kill and 50 per cent inhibition of glucose and lactate oxidation are summarised in Table IV.

TABLE IV

EFFECT OF DETERGENTS ON VIABILITY AND SUBSTRATE OXIDATION USING *E. coli*

Detergent	Concentration to produce		
	50 per cent kill	50 per cent inhibition glucose oxidation by intact cells	50 per cent inhibition of lactate oxidation by intact cells
1	40 μg./ml.	27 μg./ml.	—
2	95 ,,	88 ,,	180 μg./ml.
3	120 ,,	110 ,,	—
4	140 ,,	140 ,,	—
5	260 ,,	225 ,,	—

1. 1-*n*-Hexadecylpyridinium chloride (Ceepryn). 2. Benzalkonium. 3. Cetrimide. 4. *N*-(Nonyl-naphthyl-methyl)-pyridinium chloride (Emcol 888). 5. *N*-(Lauryl-colamino-formyl-methyl)-pyridinium chloride (Emulsept).

The figures for the viable count may be low as dilutions were plated out into Endo's agar and counts made after 24 hours, while it is customary to count after 48 or even 36 hours incubation, and no attempt was made to neutralise the cationic detergent or to overcome the clumping that occurs with this class of antiseptic. With these reservations, the Table shows a relation between killing and glucose oxidase inhibition. The detergent: bacterial nitrogen ratio for 50 per cent inhibition of lactate oxidation to benzalkonium was 180 μg./mg. N for intact cells, a higher figure than the corresponding value for glucose (88 to 90 μg./mg. N). A "considerable stimulation" of lactate oxidation by sub-bactericidal amounts of this detergent was reported. No actual figures were given for the extent of stimulation, nor was this observation discussed. The arginine decarboxylase activity of intact cells was found to be remarkably resistant to benzalkonium, and at certain detergent concentrations marked stimulation of its activity noted.

In this instance the arginine decarboxylase activity persists and is even stimulated at bactericidal concentrations of the detergent; this stimulation was not shown to such a marked extent with a cell-free arginine decarboxylase. The authors interpreted the stimulation to be due to an increase in the permeability of the intact bacterial cell to the substrate, caused by the benzalkonium. A lactic oxidase preparation which catalysed the oxidation by molecular oxygen of lactic acid to pyruvic acid was prepared by grinding the cells for 3 hours in a Booth-Green mill and centrifuging the resulting slurry after diluting with water. It was concluded that the specific inhibition of detergent sensitive enzymes can account for the metabolic inhibition, cell death and increased permeability observed in bacteria with bactericidal amounts of cationic detergents.

It is difficult to see how metabolic inhibition and cell death can be accounted for by the fact that a lactic oxidase is inhibited by detergents to the same extent unless this reaction is shown to be essential for the metabolism of the cell. It is even more difficult to see how this enzyme sensitivity can account for the increased permeability shown although the stimulated lactate oxidation by intact cells in the presence of subinhibitory concentrations may be attributable to a change in the permeability of the cell to substrate or coenzyme. This is the reason given for stimulated arginine decarboxylase activity.

Knivett[91] while studying the catabolism of arginine by washed suspensions of *Str. faecalis* noted that despite the complete disappearance of this amino acid only 70 to 80 per cent could be accounted for by the known products of the reaction, which were postulated to be ornithine, carbon dioxide and ammonia. Ultimately, citrulline was found in the reaction mixture and this accounted for the discrepancy. Cell-free preparations or acetone or cetrimide treated cells were found to convert arginine to citrulline and carbon dioxide. With cetrimide-treated cells the reaction is rapid and proceeds to completion. Citrulline was found to be attached very slowly to intact cells. If adenosine triphosphate is added to the reaction system, cetrimide and acetone treated cells can then convert citrulline to arginine, carbon dioxide and ammonia.

Postgate[92] has demonstrated the leak of a cytochrome component from *Desulphovibrio desulphuricans*, on treatment of the cells with cetrimide.

Conclusion

Until recent years so little was known about the antibacterial action of phenols and their chlorinated and nitrated derivatives that it was described in general terms as non-specific, or they were even less helpfully known as general protoplasmic poisons or protein precipitants.

It was the application of what is, in terms of modern methods, the relatively crude methods of micro-respiration which provided the clue to the precise mechanism of action of the substituted phenols. In turn these methods have been applied to the cationic surface-active compounds. It may be expected that more modern methods will prove equally successful. In particular some of the problems of cell interface reactions may be expected to resolve to the more physico-chemical methods which have been applied to single-cell studies, and it may be that interference and phase-contrast microscopy will find a direct application. There is scope for systematic investigation of the mode of action of the newer antiseptics now being introduced into medicine, and it can be expected that interesting and unsuspected mechanisms may be discovered.

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