BACTERIAL LYSIS

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Lysis by Physical and Chemical Methods

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The term lysis as applied to bacteria may be taken as referring to one or more processes; it has been used to describe the optical clearing of a bacterial suspension (Salton, 1957), and also to describe leakage of small molecular weight materials without optical clearing. The first usage is probably more rigorous and is analogous to the use of the term in haemolysis. For the present communication, however, it is proposed to use the term to cover leakage as well as optical clearing. The term is thus taken to cover what are called protytic phenomena (e.g. K^+ leakage) in erythrocytes.

To separate for discussion lysis due to 'chemical' or 'physical' agents is also to some extent arbitrary. One may presume that even bacteriophages operate by the laws of physics and chemistry, but it is convenient to focus attention on more or less non-specific methods of lysis. Under physical methods of lysis, one may include heating, freezing, osmosis and shaking, etc., and under chemical lysis the effect of acids and bases, detergents, and the almost non-specific reagents such as alcohols, ether, etc. It is hardly necessary to point out that a physical process such as freezing or a chemical reaction involving detergents, for example, may in fact lead to lysis by initiating a biochemical process such as enzymic autolysis, and that in particular cases no clear distinction between biochemical, physical or chemical lysis is always possible. The present discussion will not consider physical lysis in any detail beyond pointing out certain obvious explanations such as phase changes in membranes on cooling, heat denaturation of proteins, and mechanical fragility on shaking.

In discussing chemical lysis, attention will be focused on the lytic effects of ionic detergents and of non-ionic soluble molecules such as butanol.

The fact that a very large amount of work has been carried out on the lysis of red blood cells makes it convenient to compare and contrast the lytic reactions of bacteria and red cells. In the first place it is useful to compare the structure and activities of the red cell and bacteria. The most obvious difference between the two classes of cell is the absence of a cell wall around the erythrocyte. Functionally the cell wall protects bacteria against osmotic fluctuations not met by erythrocytes. Mitchell (1954) and Mitchell & Moyle (1956*a*) have proved that the protoplasmic membrane of bacteria acts as an osmotic barrier, and that about 70 % of the intracellular water is osmotically available. Weibull (1956) and McQuillen (1956) have shown that bacterial protoplasts act as osmometers and may be lysed by shaking or other means to produce a ghost which can be identified with the protoplast membrane. The protoplast membrane may be identified with the small-particle fraction of disrupted cells and contains about 40 % protein and 20 % lipid in the case of *Staphylococcus aureus* (Mitchell & Moyle, 1956*a*). The lipid of the protoplast membrane is likely to be predominantly phospholipid. The same conclusion also applies to *Micrococcus lysodeikticus* (Gilby & Few, 1957). The exchange of phosphate ions across the membrane is of the exchange diffusion type in resting cells of *Escherichia coli* and *S. aureus*, with little net transport (Mitchell & Moyle, 1956*b*). These properties, and the lack of mechanical rigidity of the protoplast membrane (Mitchell & Moyle, 1956*a*) show great similarities between bacterial protoplasts and red cells (Ponder, 1948). On the other hand, the protoplast membrane is the site of many enzymes, whereas only acetylcholine esterase has been definitely located in the red cell stroma (Paléus, 1947). In addition, the overall lytic properties of bacteria depend on the growth phase, the strength of the cell wall, degree of metabolic activity, aerobiosis, etc. Mitchell & Moyle (1956*b*) have given strong thermodynamic reasons for regarding the protoplast membrane as an ordered lipoprotein layered structure similar to that proposed by Stein & Danielli (1956), which is permeable to phosphate by a process of reversible denaturation. The red cell membrane may also be regarded in this way (Adair, 1956). Electrophoretic data shows that the erythrocyte exterior surface is probably lipid, with a predominance of charged phosphate groups (Seaman & Pethica, 1957), whereas the protoplast of *M. lysodeikticus* has an exterior surface of a protein-type (Seaman, G. V. F., unpublished results, 1957). This difference may be related to the adhesion of the protoplast to the cell wall. Further differences between erythrocytes and bacterial protoplast membranes are that no cholesterol is found in bacterial membranes (Gilby & Few, 1957), and that whereas erythrocytes are more permeable to anions than cations, the reverse is likely to be true for some bacteria, such as *S. aureus* (Mitchell & Moyle, 1956*b*).

This brief account of the comparative properties of red cell and bacterial membranes enables certain conclusions to be drawn about the mechanism of the lytic action of detergents on bacteria. There is abundant evidence that the ionic detergents are bactericidal through their effect on the cytoplasmic

membrane (Schulman, Pethica, Few & Salton, 1955; Salton, 1957). The leakage of small molecules (e.g. nucleotides) induced by ionic detergents is in general more easily brought about with Gram-positive than with Gram-negative bacteria. This difference is related to differences in the cell walls of Gram-positive and Gram-negative bacteria. The Gram-negative organisms so far studied have a high lipid content in the cell walls, whereas the cell walls of Gram-positive organisms have a low lipid content (Salton, 1952, 1953). Salton (1957) suggested that the screening effect of the cell-wall lipids, which will react with detergents, protects the cytoplasmic membrane of Gram-negative organisms. The effect of detergents in dispersing the cell walls themselves, and thus permitting optical clearing of the bacterial suspension, has also been discussed by Salton (1957). After the initial rapid effect of the detergents in causing leakage of small molecules, a slower process which terminates in the complete lysis of the cells is often observed. This process is ascribed to the action of autolytic enzymes on the disorganized or dead cell (Salton, 1951; Dawson, Lominski & Stern, 1953). In the case of the Gram-negative *Proteus vulgaris* and *Pseudomonas viscosa* the full lysis effected by sodium dodecyl sulphate and Teepol is probably due to a direct action of the detergent on the lipid-rich cell wall (Salton, 1957; Lominski & Lendrum, 1942). The detergent in this case will act strictly as a detergent, 'cleaning' the membrane of the lipids. Under these circumstances the cytoplasmic membrane is also destroyed leaving no ghost (Salton, 1957). This destruction of the ghost is also found when dodecyl sulphate lyses erythrocytes (Pethica, B. A., unpublished results, 1957). The ionic detergents when not acting directly to dissolve the wall, may increase the rate of autolysis by removal of inhibitors of autolysis. It may also be noted that detergents affect enzymes markedly. Sodium dodecyl sulphate, for example, inhibits succinic oxidase, urease, pancreatic lipase, invertase, papain and amylase, but stimulates the action of trypsin on native proteins (Wills, 1954). Sodium dodecyl sulphate also inhibits acetylcholine esterase (Anderson, 1954), but has little effect on serum choline esterase or cytochrome oxidase (Wills, 1954). Bacterial walls contain few, if any, enzymes (Weibull, 1956). Detergents acting on the enzyme-rich cytoplasmic membrane could thus easily allow contact and reaction between the wall and enzymes which normally are kept structurally away from the wall.

The actual site of reaction of the ionic detergents on the protoplast membrane is not definitely known. Detergents are known to denature proteins (Putnam, 1948), inhibit enzymes (Wills, 1954) and interact with lipids at interfaces (Schulman & Rideal, 1937; Pethica, 1955). The haemolytic effect of a range of ionic detergents is parallel to their ability to penetrate monolayers of cholesterol (Pethica & Schulman, 1953; Schulman *et al.* 1955; Pethica & Anderson, 1953). Cholesterol is thus a likely primary site of attack for ionic detergents acting on red cells. Gilby & Few (1957) showed with the dodecyl series of ionic detergents, that the lytic action against the protoplast of *Micrococcus lysodeikticus* was in the order of head groups:

This sequence is contrary to the haemolytic series. Anderson & Pethica (1955) showed that dodecylamine interacts more effectively than dodecyl sulphate with lecithin monolayers, which is in the correct order for protoplast lysis. Until more evidence is forthcoming it may be tentatively assumed that the ionic detergents interact principally with phospholipids of the bacterial membrane. This suggestion is reinforced by the fact that dodecyl trimethyl ammonium bromide interacts poorly with the protein bovine plasma albumin, whereas sodium dodecyl sulphate interacts strongly with this and other proteins (Few, Ottewill & Parreira, 1955; Putnam, 1948). Physico-chemically the effect of these detergents is to break down irreversibly a lipoprotein structure either by removal (detergency) of important components of the membrane or by disordering the structure by penetration.

The ionic detergents may be expected to engage in fairly specific reactions with membrane components because of the high energies of ion-dipole and ion-ion interactions of detergents (Pethica, 1955; Goddard & Schulman, 1953). In the case of non-ionic surface active agents this specificity will be much lower. It was shown for haemolysis that non-ionic surface active agents of various types cause lysis when the surface pressure of the solution (lowering of the air/solution surface tension) was >34 dynes/cm. For butanol the concentration at which this surface pressure is attained is 0.39 M. Mitchell (1953) showed that leakage of phosphate occurred from *Staphylococcus aureus* above a critical concentration of 0.4 M. Dr Mitchell has now shown that the same critical concentration of butanol causes leakage in *Micrococcus lysodeikticus*, *Sarcina lutea* and *Escherichia coli*. It is also known that irreversible blocking of the frog sciatic nerve occurs at a butanol concentration above 0.4 M (Skou, 1954). A similar concentration of butanol breaks down the ram spermatozoon membrane (Mann, T., private communication). Kaplan (1954) showed that yeast catalase was removed from an intracellular surface structure by butanol at 0.38 M and by a range of aldehydes, ketones and alcohols giving a surface tension lowering of about 32 dynes/cm. These effects in a range of biological systems suggest that a common membrane link or structure is being altered by butanol and similar agents (Pethica, 1956). It was suggested for red cells that the critical surface pressure for lysis indicated a maximum two-dimensional free energy beyond which the surface became occupied by the non-ionic surface active agent with collapse of the membrane (Pethica & Schulman, 1953). Other explanations of the effect of alcohols are possible. Stein & Danielli (1956) suggested that butanol can act as a hydrogen-bonding agent in disrupting the hydrogen-bonded structure of the membrane of a red cell. The similarity of the action of butanol in this range of lytic and irreversible blocking phenomena calls to mind the work of Morton (1953, 1954), who showed that butanol was able to release an alkaline phosphatase from association with a lipoprotein in milk and calf intestinal mucosa. Regarding the membrane as a lipoprotein complex, the effect of butanol and similar non-ionic surface active agents may be ascribed to breaking of lipid-protein (enzyme) linkages, either by acting as a hydrogen-bond breaker or by competing for the ionic components of ion-dipole linkages. The effect of butanol

and similar compounds in narcosis may be regarded as an early, reversible stage of membrane breakdown. Narcosis is essentially reversible and occurs at lower concentrations than lysis or irreversible alteration of membranes. The question as to whether narcotics act on membranes or on intracellular lipids has often been debated (Dethier, 1954). In the case of the red cell, the lipids of the cell are concentrated in the membrane (Ponder, 1948), and butanol and other narcotic agents may be presumed to act at the membrane. Since excitability of the nerve cell is related to the ion permeability of the nerve membrane, the narcotic and blocking action of butanol, etc., will also be at the membrane surface. Accepting the view that the permeability of biological membranes is a process involving a reversible denaturation of the membrane structure (Mitchell, 1954; Adair, 1956), the effect of low concentrations of butanol and other narcotics will be to reversibly modify the membrane structure to allow ion leakage. In red cells this is manifest as an exchange of K^+ and Na^+ without lysis, at sublytic butanol concentrations. In the nerve cell, the alteration of ion permeability at narcotic concentrations causes loss of excitability. It would be interesting to observe whether ion leakage of K^+ occurs at narcotic concentrations with bacteria. At higher concentrations, the alteration of the membrane becomes critical leading to haemolysis (leakage of haemoglobin), irreversible blockage of nerve cells, breakdown of the specific phosphate exchange mechanism in bacteria and leakage of nucleotides, etc. It is likely that the effect of pH and other factors (e.g. sucrose concentration) on the critical lytic concentrations of butanol will give valuable information on cell-membrane bonding.

In concluding this contribution to the discussion it would seem permissible for a physical chemist to make a few remarks on the interpretation of a number of experimental variables which are important in studies of bacterial and other lysis. The variables in question are pH value, salt concentration and temperature. These three variables should be regarded as interdependent. The importance of the pH value needs no emphasis, but it is often overlooked that measurements of pH carried out in the conventional manner are not sufficient in themselves to define the effective concentration of hydrogen ions at biological membranes. At any surface which carries ionic charges the effective concentration of hydrogen ions near the surface is a function of the pH value of the bulk of the solution (as measured on a pH meter) and of the surface potential. Assuming a Boltzmann distribution and no solvation effects, the pH value near a surface (pH_s) is given by:

$$pH_s = pH + \frac{e\psi}{2.3kT}, \quad (1)$$

where ψ is the surface or double layer potential, e the proton charge, k the Boltzmann constant and T the absolute temperature (Danielli, 1937; Hartley & Roe, 1940; Betts & Pethica, 1956). The value of ψ depends on the surface-charge density and the salt concentration for a given pH value. The term 'salt concentration' is used in preference to 'ionic strength' here because ionic strength is a property of a salt solution relevant to ionic concentrations in

three dimensions. Near a surface, the most important concentration in defining the colloid properties of the surface and the value of ψ is the concentration of ions of opposite sign to the surface charge. To keep ionic strength constant is to treat the system as three dimensional. For a surface system the salt concentration should be adjusted to keep the counter ions at an effective constant concentration. This may be calculated in the absence of specific binding from the Gouy theory (Verwey & Overbeek, 1948). To give an example, 0.1 M-NaCl is of equal ionic strength with 0.033 M- Na_2SO_4 , but has an equivalent Gouy concentration of 0.025 M for a positive surface and 0.05 M for a negative surface at low-charge densities. Variations of bulk pH value which are accompanied by changes in salt concentration as discussed above, thus alter the effective pH values at membranes, etc., as a function of two variables, and not of one. The importances of differences in pH values between biological surfaces and bulk solutions may be realized from the fact that for a close-packed fatty acid monolayer, only a small degree of ionization takes place at $\text{pH}=4.8$ (the nominal half-ionization pH value) in 0.1 M-NaCl. Dilution of the NaCl at constant pH depresses the ionization in the surface still further (Betts & Pethica, 1956). The importance of surface pH has been discussed by Frazer (1957) and by McLaren (1957) in relation to enzyme reactions at interfaces. The relevance of surface pH value to oxidation-reduction reactions involving charged thiol compounds is discussed by Danielli & Davies (1951). Surface pH has been taken into account in interpreting the ionization of charged groups at the surface of human erythrocytes (Seaman & Pethica, 1957) and of *Escherichia coli* (Davies, Haydon & Rideal, 1956).

The effect of temperature on biological reactions is often interpreted in terms of activation energies. It is well known that activation energies for enzyme reactions may include the heat of ionization of the reactive group on the enzyme when this is weakly ionized, due to the change of pK of the active groups with temperature (Gutfreund, 1955). This same consideration also applies to reactions at charged membranes. In addition, a membrane charge which is not directly concerned in a reaction will also influence the rate of a reaction if the reagent is charged. Thus activation energies for charged molecules permeating a membrane will be related to the intrinsic activation of the specific permeation reaction and to the change of potential at the membrane resulting from the change of membrane charge with temperature. An increased negative membrane-potential will decrease the apparent activation energy of a positive molecule and increase the apparent activation for a negative molecule. We may note that Mitchell's (1954) calculation of the activation energy for phosphate transfer in *Staphylococcus aureus* may be in error for this reason, although the probable magnitude of the error is insufficient to alter his interpretation of the activation energy. Once again the importance of salt concentration should be borne in mind, in that the activation energies for charged molecules will also depend, in general, on the effect of salts on the surface potential of the membrane.

REFERENCES

- ADAIR, G. S. (1956). Discussions of the Faraday Society, no. 21, p. 285.
- ANDERSON, P. J. (1954). Doctorate Thesis, University of Birmingham.
- ANDERSON, P. J. & PETHICA, B. A. (1955). Surface chemistry of synthetic lecithin. *2nd International Conference on the Biochemical Problems of Lipids (Ghent)*, p. 24.
- BETTS, J. J. & PETHICA, B. A. (1956). The ionisation characteristics of monolayers of weak acids and bases. *Trans. Faraday Soc.* **52**, 1581.
- DANIELLI, J. F. (1937). Relations between surface pH, ion concentrations and interfacial tensions. *Proc. Roy. Soc. B*, **122**, 155.
- DANIELLI, J. F. & DAVIES, J. T. (1951). Reactions at interfaces in relation to biological problems. *Advanc. Enzymol.* **11**, 35.
- DAVIES, J. T., HAYDON, D. A. & RIDEAL, Sir ERIC K. (1956). Surface behaviour of *Escherichia coli*. *Proc. Roy. Soc. B*, **145**, 375.
- DAWSON, I. M., LOMINSKI, I. & STERN, H. (1953). An electron microscope study of the action of cetyl trimethyl ammonium bromide (CTAB) on *Staphylococcus aureus*. *J. Path. Bact.* **66**, 513.
- DETHIER, V. G. (1954). The physiology of olfaction in insects. *Ann. N.Y. Acad. Sci.* **58**, 139.
- FEW, A. V., OTTEWILL, R. H. & PARREIRA, H. C. (1955). Interaction between bovine plasma albumin and dodecyl trimethyl ammonium bromide (DTAB). *Biochim. biophys. Acta*, **18**, 136.
- FRASER, M. J. (1957). Behaviour of proteins at interfaces. *J. Pharm. Lond.* **9**, 497.
- GILBY, A. R. & FEW, A. V. (1957). Surface chemical studies on the protoplast membrane of *Micrococcus lysodeikticus*. *2nd International Conference on Surface Activity (London)*, **4**, 262. London: Butterworths.
- GODDARD, E. D. & SCHULMAN, J. H. (1953). Molecular interaction in monolayers. I. Complex formation. *J. Colloid Sci.* **8**, 309.
- GUTFREUND, H. (1955). Characterisation of the catalytic site of trypsin. *Trans. Faraday Soc.* **51**, 441.
- HARTLEY, G. S. & ROE, J. W. (1940). Ionic concentrations at interfaces. *Trans. Faraday Soc.* **36**, 101.
- KAPLAN, J. G. (1954). The alteration of intracellular enzymes. II. The relation between the surface and biological activities of altering agents. *J. gen. Physiol.* **38**, 197.
- LOMINSKI, I. & LENDRUM, A. C. (1942). Effect of surface active agents on *B. proteus*. *J. Path. Bact.* **54**, 421.
- McLAREN, A. D. (1957). Concerning the pH dependence of enzyme reactions on cells, particulates and in solution. *Science*, **125**, 697.
- McQUILLEN, K. (1956). Capabilities of bacterial protoplasts. *Symp. Soc. gen. Microbiol.* **6**, 127.
- MITCHELL, P. (1953). Transport of phosphate across the surface of *Micrococcus pyogenes*: nature of the cell inorganic phosphate. *J. gen. Microbiol.* **9**, 273.
- MITCHELL, P. (1954). Transport of phosphate through an internal barrier. *Symp. Soc. exp. Biol.* **8**, 254.
- MITCHELL, P. & MOYLE, J. (1956*a*). Osmotic function and structure in bacteria. *Symp. Soc. gen. Microbiol.* **6**, 150.
- MITCHELL, P. & MOYLE, J. (1956*b*). Permeation mechanisms in bacterial membranes. *Disc. Faraday Soc.* no. 21, p. 258.
- MORTON, R. K. (1953). Alkaline phosphatase of milk. I. Association of the enzyme with a particulate lipoprotein complex. *Biochem. J.* **55**, 786.
- MORTON, R. K. (1954). Purification of alkaline phosphatases of animal tissues. *Biochem. J.* **57**, 595.

- PALÉUS, S. (1947). Localisation of the specific cholinesterase in human blood. *Arch. Biochem.* **12**, 153.
- PETHICA, B. A. (1955). The thermodynamics of monolayer penetration at constant area. *Trans. Faraday Soc.* **51**, 1402.
- PETHICA, B. A. (1956). *Disc. Faraday Soc.* no. 21, p. 286.
- PETHICA, B. A. & ANDERSON, P. J. (1953). The role of the phospholipids and cholinesterase in the haemolytic reaction. *1st International Conference on the Biochemical Problems of Lipids (Brussels)*, p. 129.
- PETHICA, B. A. & SCHULMAN, J. H. (1953). The physical chemistry of haemolysis by surface active agents. *Biochem. J.* **53**, 177.
- PONDER, E. (1948). *Haemolysis and Related Phenomena*. London: J. and A. Churchill.
- PUTNAM, F. W. (1948). The interactions of proteins and synthetic detergents. *Advanc. Protein Chem.* **4**, 79.
- SALTON, M. R. J. (1951). The adsorption of cetyl trimethyl ammonium bromide by bacteria, its action in releasing cellular constituents and its bactericidal effects. *J. gen. Microbiol.* **5**, 391.
- SALTON, M. R. J. (1952). The nature of cell walls of some gram positive and gram negative bacteria. *Biochim. biophys. Acta*, **9**, 334.
- SALTON, M. R. J. (1953). Composition of the cell walls of some gram positive and gram negative bacteria. *Biochim. biophys. Acta*, **10**, 512.
- SALTON, M. R. J. (1957). The action of lytic agents on the surface structures of the bacterial cell. *2nd International Conference on Surface Activity (London)*. **4**, 245. London: Butterworths.
- SCHULMAN, J. H., PETHICA, B. A., FEW, A. V. & SALTON, M. R. J. (1955). The physical chemistry of haemolysis and bacteriolysis by surface active agents and antibiotics. *Progr. Biophys.* **5**, 41.
- SCHULMAN, J. H. & RIDEAL, E. K. (1937). Molecular interaction in monolayers. I. Complexes between large molecules. II. Action of haemolytic and haemagglutinating agents on lipoprotein monolayers. *Proc. Roy. Soc. B*, **122**, 29, 46.
- SEAMAN, G. V. F. & PETHICA, B. A. (1957). The electrophoretic characteristics of the human normal and sickle erythrocyte. *2nd International Conference on Surface Activity (London)*. **4**, 277. London: Butterworths.
- SKOU, J. C. (1954). Local anaesthetics. I-VI. *Acta pharm. tox., Kbh.* **10**, 281, 292, 297, 305, 317, 325.
- STEIN, W. D. & DANIELLI, J. F. (1956). Structure and function in red cell permeability. *Disc. Faraday Soc.* no. 21, p. 238.
- VERWEY, E. J. W. & OVERBEEK, J. TH. G. (1948). *Theory of the stability of lyophobic colloids*. Amsterdam: Elsevier.
- WEIBULL, C. (1956). Bacterial protoplasts. Their formation and characteristics. *Symp. Soc. gen. Microbiol.* **6**, 111.
- WILLS, E. D. (1954). Effect of anionic detergents and some related compounds on enzymes. *Biochem. J.* **57**, 109.