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The effect of antibiotics on bacteria under hyperbaric conditions

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The sensitivity of selected bacteria to a range of antibiotics was tested under hyperbaric conditions used in saturation diving. The effect of hyperbaric helium and oxygen (heliox) on antibiotic stability and on induction of β-lactamase was also determined. Increased resistance to penicillin (up to 23%) was shown by *Staphylococcus aureus* and to gentamicin (up to 46%) and rifampicin (up to 18%) by *Escherichia coli* and *Salmonella typhimurium* at 36 and 71 bar pressure. Exposure to 71 bar heliox did not affect antibiotic activity but increased the production of β-lactamase in inducible *S. aureus* and *Bacillus subtilis* and production of β-galactosidase in inducible *E. coli*. Increased resistance to antibiotics in saturation diving conditions can be attributed in some cases to the influence of hyperbaric pressure on induction mechanisms in bacteria. The experimental system devised for this work is suitable for more detailed examination of the influence of hyperbaric stress on antibiotic resistance and of its effect on induction mechanisms in general.

Introduction

Saturation diving is a technique used by the Navy and by commercial organisations to increase the depth at which divers work and the length of time which can be spent at such depths. The divers live in chambers pressurised to between 5 and 70 bar (1 bar = 1 atmosphere = 10^5 Pascal) containing an atmosphere of helium and oxygen (heliox = helium containing oxygen at a partial pressure of 0.4 bar) which saturates their tissues. They may remain under these hyperbaric conditions for weeks at a time and are conveyed to and from their deep water work area in pressurised vessels (Walder, 1981).

The confined living conditions and elevated humidity and temperature experienced within a saturation complex can bring about changes in the normal microflora of the body (Jones & Davis, 1978) and increase the risk of infection (Hojyo-Tomoka, Marples & Kligman, 1973; Alcock, 1977; Nichols, Goad & Page, 1983; Victorov *et al.*, 1992). The most commonly encountered infections associated with saturation diving are otitis externa, involving pseudomonads (Thalman, 1974; Alcock, 1977; Strauss & Dierker, 1987) and staphylococcal and fungal infections of the skin (Nichols, G., personal communication). The return of divers to normal atmospheric pressure, by decompression, can take several days. Antimicrobial chemotherapy, when required,

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must therefore be administered in the hyperbaric heliox atmosphere of the saturation complex.

A number of reports suggest that susceptibility of bacteria to antibiotics is affected by exposure to the stress of hyperbaric conditions (Gottlieb, 1971; Schlamm, 1972; Kenward, Alcock & McKay, 1984), either by increasing or decreasing resistance. However, much of the previous experimental work in this field has been carried out using bacteria, gas mixtures and pressures not directly relevant to normal saturation diving conditions. Alternatively experimental systems such as solid media have been used (Kenward *et al.*, 1984), which are unsuitable for subsequent investigation of the mechanisms responsible for pressure-induced changes in antibiotic susceptibility.

This paper describes the effects of a range of antibiotics on the growth of bacteria during exposure to high gas pressures. The bacteria and experimental conditions used were chosen to reflect those encountered in saturation diving. The experimental system developed for the work was designed to facilitate subsequent investigations of the mechanisms involved. Bacteria known to have caused infection during diving were chosen for study, as well as strains used in previous studies on the effects of pressure (Schlamm, Perry & Wild, 1974). The antibiotics used were selected to cover a range of cellular target sites and modes of action and not because they are considered to be of therapeutic value in infections caused by the test organisms.

The heliox composition (maintaining oxygen partial pressure at 0.4 bar) and pressures employed (up to 71 bar absolute, equivalent to 700 m depth) are those used in diving operations.

Materials and methods

Bacterial strains

The bacteria used were *Enterococcus faecalis* NCTC 775, *Escherichia coli* NCTC 10216, *Pseudomonas aeruginosa* NCTC 6750, *Salmonella typhimurium* NCTC 74, *Staphylococcus aureus* ATCC 13301 *Streptococcus pneumoniae* NCTC 10319 and *Streptococcus pyogenes* NCTC 8884.

β-Lactamase-inducible bacterial cultures of *S. aureus* ATCC 13301 and *Bacillus subtilis* NCTC 6431 were used together with the constitutive producer *S. aureus* NCIMB 11195 to investigate β-lactamase production under hyperbaric conditions.

Stock cultures were held in a freeze dried state and cultured on Brain Heart Infusion (BHI) agar or broth (Unipath, UK) when required.

Experimental work in hyperbaric conditions

Cylindrical steel pressure vessels were used with an internal diameter of 124 mm and height of 175 mm. Each vessel held 12 bacterial cultures or test systems.

Tubes containing the cultures or test systems were placed into a pressure vessel which was then closed, evacuated and refilled with the appropriate experimental gas mixture 5 times to achieve the atmosphere required for work at 1 bar. For work at 36 or 71 bar (absolute) the closed vessel was evacuated and then filled with the experimental gas mixture to a pressure of 10 bar. The pressure was released immediately and the vessel recompressed to 10 bar. The pressure was released again and the vessel repressurised

this time to the experimental level. These procedures were necessary to purge the system of air and to achieve the gaseous environment required.

The pressure vessels used have a high heat capacity and were therefore brought to incubation temperature before experimental work began. Temperatures inside the vessel were monitored during pressurisation and decompression by means of probes suspended in the atmosphere and the growth medium.

The composition of gas mixtures inside each vessel was analysed by gas chromatography after incubation. This was done to check that nitrogen and carbon dioxide levels had not become excessive and that the required concentration of oxygen had been maintained. The percentages of oxygen required to maintain a partial pressure of 0.4 bar in heliox mixtures at the experimental pressures of 36 and 71 bar were 1.25% and 0.62% respectively.

Determination of antibiotic sensitivity

Minimum inhibitory concentration (MIC) was determined by inoculating sets of tubes containing an appropriate concentration range of the antibiotic under test in BHI broth. The inoculum used as 0.02 mL of a 1/100 dilution of a 12 h culture also in BHI broth. The tubes were incubated at 37°C for 18 h in a pressure vessel containing heliox and set up as described above.

The antibiotics used were colistin, erythromycin, gentamicin, nalidixic acid, oxytetracycline, penicillin G, rifampicin and vancomycin (Sigma, UK), sterilised by filtration. Each batch of antibiotic solution was tested to ensure consistency of activity by determination of MIC for *E. coli* and *S. aureus* in air at 1 bar. All MIC determinations were carried out in triplicate. Also, one triplet of tubes were incubated at 1 bar and a second triplet at the experimental pressure. Dilutions of all antibiotics used were prepared in duplicate in BHI broth.

Determination of antibiotic stability under hyperbaric conditions

Dilutions of those antibiotics to which bacteria showed reduced susceptibility under pressure were prepared in duplicate in BHI broth. One set of dilutions was held at 37°C in heliox at 71 bar and the duplicate set at 1 bar, for 18 h. Both sets were then inoculated with the appropriate bacteria and incubated in air at 1 bar for 24 h. The resulting MICs were compared to determine the effect of holding antibiotics in hyperbaric conditions.

Determination of β-lactamase production under hyperbaric conditions

Constitutive and inducible β-lactamase producers as well as the pure enzyme were used in this part of the work. The inducers used were methicillin (Sigma, UK) 0.2 mg/L and 6-aminopenicillanic acid (6-APA) (Sigma, UK) 100 mg/L. Methicillin was selected in order to allow comparison with the work of Wild (1976). The second inducer, 6-APA, was chosen because it is a good inducer of β-lactamase but, unlike alternatives such as penicillin G, has poor antibiotic activity (Lemke & Brannon, 1972). It could therefore be used at a relatively high concentration to compensate for some degradation by β-lactamase, and to maintain induction throughout the experiment without inhibiting the test culture. At 12 h culture of the test organism was diluted 1 in 10 in BHI broth containing one of the inducers. One set of cultures was incubated at 71 bar and a

duplicate set at 1 bar. Following incubation cells were removed from the cultures by centrifugation. The concentrations of β-lactamase in the supernatant was determined using a method adapted for use in a pressure vessel (Sargent, 1968).

β-Lactamase activity was recorded in Perret units (Perret, 1954). One Perret unit is the quantity of enzyme hydrolyzing one mole of penicillin G per hour at pH 6.5 and 30°C. A calibration curve was obtained on the basis of Perret's observation that 1 ml of 0.0166 N sodium thiosulphate is equivalent to 2 moles of penicillin G destroyed by β-lactamase. Absorbance data obtained from the β-lactamase assay could therefore be converted to penicillinase units.

Statistical analysis

Analysis of variance (ANOVA) were performed using the MINITAB program of the University of Minnesota, USA.

Results

Culture conditions

The composition of the gas within the pressure vessel after incubation was within the limits acceptable for saturation diving operations as shown in Table I.

The temperature of the culture medium dropped from 35°C to 33.2°C during compression to 71 bar with heliox. Approximately 20 min after reaching experimental pressure it rose steadily towards the target incubation temperature of 37°C.

Effect of hyperbaric conditions on antibiotic susceptibility

Changes in bacterial resistance to antimicrobial agents, brought about by hyperbaric conditions, are presented in Table II as the difference between MICs at 1 and 71 bar. Some of the differences in MIC appear large but were not significant ($P = 0.05$) because of wide variation in the data from which mean values at both pressures were determined. The bacteria/antibiotic combinations underlined showed a significantly altered MIC ($P = 0.05$) at 71 bar from that at 1 bar. All other combinations gave MICs at 36 and

Table I. Composition of heliox gas mixture in the pressure vessel after incubation

Gas	Pressure 36 bar % of total	71 bar % of total
Helium	98.638	99.387
Oxygen	1.258	0.613
Nitrogen	0.096	0.000
Carbon Dioxide	0.008	0.000
pO_2 after incubation (Calculated from % O_2)	0.450 bar	0.435 bar
Desired pO_2	0.400 bar	0.400 bar

Table II. The difference between MICs at 1 bar and at 71 bar (mg/L)

	E. coli	*S. typhimurium*	*P. aeruginosa*	*S. aureus*	*S. faecalis*	*S. pneumoniae*	*S. pyogenes*
Penicillin	V	−0.2	NS	+7.0	0	−0.001	0
Gentamicin	+0.34	+0.4	−0.2	NT	NS	NS	NS
Colistin	−0.02	0	−0.02	NS	NS	NS	NS
Nalidixic acid	+0.3	−0.2	NS	NT	NS	NS	NS
Vancomycin	NS	NS	NS	−0.1	−0.4	0	−0.02
Rifampicin	+2.0	+2.0	−4.0	−0.001	+0.25	+0.03	−0.02
Erythromycin	V	−1.0	V	V	−0.42	0	0
Oxytetracycline	0	−0.2	−2.2	NT	−0.07	−0.09	−0.02

NS, MIC was greater than the arbitrary limit of 100 mg/L employed in this study.
NT, Not tested.
V, Variability of the data was too large for a meaningful value to be determined under the conditions employed in the assay.
The underlined values are statistically significant differences in MIC ($P = 0.05$).

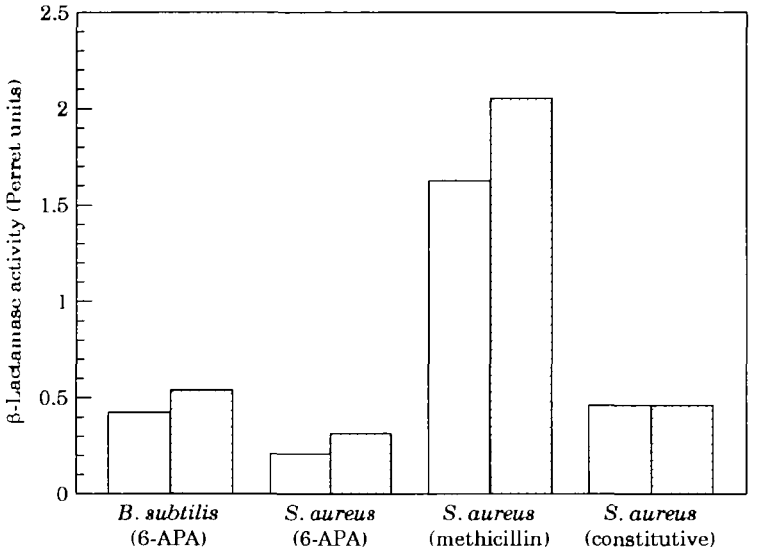

Figure Production of β-lactamase by *B. subtilis* following induction with 6-APA, production of β-lactamase by *S. aureus* following induction with 6-APA and methicillin and production of β-lactamase by a constitutively producing strain of *S. aureus*. □, 1 bar; ■, 71 bar E

71 bar which were equal to or were not significantly different ($P = 0.05$) from those at 1 bar.

Stability of antibiotics under hyperbaric conditions

The antibiotics tested did not appear to be inactivated at the pressures used.

Effect of hyperbaric conditions on induction of β-lactamase

The effect of pressure on the induction of β-lactamase is shown in the Figure. Production of the enzyme by the inducible strain of *S. aureus* is increased significantly ($P = 0.05$) at 71 bar. This effect was seen whether 6-APA or methicillin was the inducer. There was no significant increase in the case of *S. aureus* NCIMB 11195, the strain constitutive for β-lactamase production.

The effect of pressure on β-lactamase production in a different inducible bacterium, *B. subtilis*, is also presented in the Figure. A significant increase in induction by 6-APA was seen when the culture was exposed to 71 bar.

Discussion

The composition and pressure of the gaseous environment produced within the experimental system reflected accurately that encountered in saturation diving complexes (Table I). The deviation in culture incubation temperature associated with gas compression at the beginning of each experiment was small and not considered to have a significant effect. The system used allowed determination of bacterial antibiotic susceptibility under realistic hyperbaric conditions. It also facilitated more detailed

investigation of susceptibility changes. It differed in these respects from the approach adopted for many previous studies. The work of Kenward *et al.* (1984) for example, employed relatively low pressure (7 bar) and agar diffusion, which is not suitable for use in more detailed follow up studies. Nichols (1978) used compressed air rather than heliox.

The results of greatest concern, in the context of saturation diving, are those which indicate an increase in resistance to an antibiotic under hyperbaric conditions. However, any change in resistance, whether increase or decrease, is significant in the investigation of mechanisms which are responsible for changes in response to elevated pressure. This work was particularly concerned with the reduction of antibiotic effectiveness under hyperbaric conditions. Three of the bacteria tested showed an increase in resistance ($P = 0.05$) to certain antibiotics under pressure (Table II). All the remaining bacteria/antibiotic combinations either showed a decrease in resistance or were unaffected, by elevated pressure.

Increased resistance in *S. typhimurium* and *E. coli* to both gentamicin and rifampicin was demonstrated in this study. Kenward *et al.* (1984) obtained a similar result except that susceptibility of *E. coli* to rifampicin at elevated pressure was not altered. This latter observation may be due to Kenward's use of a much lower pressure (7 bar) than that used in this study. The increased resistance of *S. aureus* to penicillin observed here confirms the reports of Schlamm & Daily (1973), Wild (1977) and of Nichols (1978). Although the work focused on reduction in susceptibility under pressure, the increase in susceptibility of P. *aeruginosa* to rifampicin at 71 bar is worthy of note because of the contrast with results obtained for *E. coli* and *S. typhimurium* the other Gram-negative bacteria tested, and has not been reported previously.

The results of this study did not always parallel those obtained by other workers. For example, decrease in the susceptibility of *E. faecalis* to gentamicin (Kenward *et al.*, 1984) under increased pressure was not apparent in this study. Increased resistance of *S. aureus* to penicillin reported here and by other workers (Wild, 1977; Nichols, 1978) was not seen by Kenward *et al.* (1984). Such comparisons may provide useful insights but must be treated with caution because of different bacterial strains and conditions employed. There is however a general agreement of results showing that hyperbaric conditions influence the interaction between bacteria and antibiotics.

Increased resistance to penicillin, gentamicin and rifampicin accompanied an increase in hyperbaric pressure to 36 bar. Pressurisation to 71 bar did not produce a further increase in MIC of the same magnitude as that for 36 bar. The MICs of gentamicin for *E. coli* and penicillin for *S. aureus* decreased when they were tested at 71 bar as compared with their value at 36 bar (data not shown). In all other cases shown in Table II there was only a slight further increase in the MIC. The experimental system developed in this work was used to investigate these observations in more detail.

The possibility that decreased susceptibility may be brought about by reduction in antibiotic activity associated with exposure to hyperbaric heliox was tested. Penicillin G, gentamicin and rifampicin showed no decrease in activity following exposure to hyperbaric heliox. Data obtained from susceptibility testing of bacteria in hyperbaric conditions confirmed this, as only two of seven strains tested against rifampicin and gentamicin, and one tested against penicillin, showed an increase in resistance under hyperbaric heliox (data not shown). Therefore the increased resistance observed in this

study was not due to lability of the antibiotics in hyperbaric conditions but to changes induced in the bacteria studied.

Wild (1977) described a study of the effect of hyperbaric pressure on the induction of β-lactamase in *S. aureus*. β-lactamase activity in a strain of *S. aureus* possessing an inducible β-lactamase was greater in cultures of *S. aureus* induced with methicillin and incubated in heliox at 68 atm than in cultures treated identically and incubated at 1 atm. We were able to reproduce this effect in our pressurised system using the same strain of *S. aureus*. The increase in levels of β-lactamase synthesis in *S. aureus* ATCC 13301 when induced with methicillin and 6-APA is shown in the Figure. In contrast β-lactamase activity in a strain of *S. aureus* constitutive for the enzyme was the same in pressurised and non-pressurised cultures (Figure). These data suggest that the induction mechanism is sensitive to hyperbaric pressure and that increased production of β-lactamase is not the result of an increase in total protein synthesis (Wild, 1977). The changes in β-lactamase activity do not represent direct effects of hyperbaric heliox on enzyme activity because activity was assayed following decompression of the assay tubes. A β-lactamase-inducible strain of *B. subtilis* was also tested to determine whether this mechanism operates in bacteria other than *S. aureus*. The same effects were seen in *B. subtilis* induced with 6-APA (Figure). The increase in β-lactamase activity here probably explains the changes in susceptibility to penicillin that we and other authors have observed in *S. aureus*. Observation of the effect in both *B. subtilis* and *S. aureus* suggests that this effect occurs more widely.

The demonstration that hyperbaric heliox affects β-lactamase synthesis in two genera of bacteria suggests that the expression of other inducible systems in bacteria may be altered by hyperbaric pressure. We have shown that there is a doubling in β-galactosidase activity, compared with that at 1 bar, after incubation for 15 min at 71 bar in heliox, in cultures of *E. coli* ML 30 induced with isopropylthiogalactoside and exposed to hyperbaric conditions (data not shown). It is therefore apparent that hyperbaric heliox can affect the expression of at least two different inducible systems, one involved in antibiotic resistance, the other in carbohydrate metabolism. Elevated pressure may therefore have a general effect on induction. This can affect antibiotic resistance in a direct way, as in the case of β-lactamase activity, but it may also influence the interaction between antibiotic and bacterium in a more subtle and complex manner. Schlamm *et al.* (1974) demonstrated a reduced lag phase in *E. coli* grown in minimal media, supplemented with iron chelates and pressurised to 68 atm with heliox. Their data indicated that the iron acquisition systems of pressurised cells were derepressed to a greater extent than the non-pressurised cells. The possibility that iron-regulated genes are affected by hyperbaric heliox implies that expression of a large number of genes may be affected by raised gas pressure. In *S. typhimurium* as many as 36 genes have been shown to be regulated by iron (Foster & Hall, 1992). This may be important during an infection because iron availability is limited by host iron-binding proteins, and negatively-regulated iron-responsive genes would be induced under those circumstances. Many virulence genes are regulated by iron (Pappenheimer & Johnson, 1936; Dubos & Geiger, 1946; Bjorn *et al.*, 1978; Bjorn, Sokol & Iglewski, 1979; Warner *et al.*, 1981; Stoebner & Payne, 1988; Goldberg, DiRita & Calderwood, 1990) together with the high-affinity iron acquisition systems, the effectiveness of which may determine the outcome of an infection (Litwin & Calderwood, 1993). Exposure to hyperbaric conditions may enhance expression of these genes during an infection. This would favour growth of the pathogen and exacerbate infections acquired during diving. These

observations suggest a need for experimental work to determine the effect of iron concentration on such characteristics as expression of iron-regulated membrane proteins in a hyperbaric environment.

In this study we also demonstrated that susceptibility to rifampicin is altered when both *E. coli* and *S. typhimurium* are compressed with heliox (Table II). Binding of rifampicin to its target site, the b subunit of RNA polymerase, inhibits initiation of mRNA transcription (Hartmann *et al.*, 1967). Another effect of rifampicin is an induction of synthesis of the b and b′ subunits of RNA polymerase encoded by the *rpo*BC genes (Fukuda & Nagasawa-Fujimori, 1983). The RNA polymerase-rifampicin complex acts as a positive effector that stimulates a 2–3 fold absolute increase in the level of transcription of *rpo*BC mRNA *in vitro*. This may provide a target for hyperbaric heliox-mediated effects. Increased synthesis of the b′ subunit may decrease susceptibility to rifampicin by making more RNA polymerase available. The increased sensitivity of *P. aeruginosa* to rifampicin and the unchanged response of Gram-positive strains tested at elevated pressure argues against induction of the *rpo*BC operon by the antibiotic as an explanation for the hyperbaric response of *E. coli* and *S. typhimurium*. However, induction of the *rpo*BC operon by rifampicin has been observed only in *E. coli* and may be unique to the Enterobacteriaceae (Nakamura & Yura, 1976).

The enzymes mediating resistance to gentamicin are constitutively expressed (Shaw *et al.*, 1993) and the observations reported here suggest that their expression would not be altered by hyperbaric pressure. The target site for gentamicin has not been identified conclusively and there appear to be multiple sites which include the cell surface, 30S ribosomes and initiation of DNA replication (Hancock, 1981). Pressure effects on susceptibility to gentamicin may be mediated via a non-specific mechanism that indirectly causes increased resistance to gentamicin. If expression of iron-regulated genes is affected by hyperbaric heliox as suggested above, then the composition of the outer membrane may be altered by enhanced expression of iron-regulated outer membrane proteins altering permeability of the membrane to gentamicin and possibly providing more sinks for the antibiotic at sites external to the cell. An alternative mechanism for decreasing susceptibility to gentamicin is interference with the uptake pathway of the antibiotic. Uptake of gentamicin occurs via an energy-dependent mechanism (Davis, 1987). Studies on the uptake of uracil by *E. coli* at 15°C and 100–300 atm of hydrostatic pressure show that it is reduced by 70% (Baross, Hanus & Morita, 1974). Pressure may cause an uncoupling of energy generation and transport systems by a compression of cells which may result in lateral movement of cellular membranes relative to each other. Mutations in *E. coli* which cause increased resistance to aminoglycosides, *hem*A, NR70, and *ubi*D, cause reduced electron transport or a reduction in membrane potential (Bryan, 1982). Pressure effects on cellular uptake mechanism may have a similar effect on the energy-dependent uptake of gentamicin and hence increase the MIC for pressurised cultures. The MIC of gentamicin measured at 36 and 71 bar was significantly elevated in both *E. coli* and *S. typhimurium*.

This study has confirmed some of the observations made by Wild (1977) and extended the number of bacterial species and inducible systems shown to be affected by hyperbaric heliox. The observation that expression of β-galactosidase is also altered by hyperbaric pressure coupled with the effects of pressure in reducing the lag phase in iron limited-cultures of *E. coli* suggest that mechanisms which regulate expression of the relevant genes are target sites for hyperbaric pressure effects. These observations have consequences in the treatment of infections under hyperbaric conditions and will enable

further, more defined studies, to be carried out on the mechanism by which hyperbaric heliox affects expression of bacterial genes. Consideration must also be given to the possibility that expression of bacterial virulence may be increased when pathogenic bacteria are pressurised. This may be another factor that explains the high incidence of infections encountered by saturation divers (Alcock, 1977).

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References

Alcock, S. R. (1977). Acute otitis externa in divers working in the North Sea: a microbiological survey of seven saturation dives. *Journal of Hygiene* **78,** 395–409.

Baross, J. A., Hanus, F. J. & Morita, R. Y. (1974). Effects of hydrostatic pressure on uracil uptake, ribonucleic acid synthesis, and growth of three obligately psychrophilic marine vibrios, *Vibrio alginolyticus* and *E. coli*. In *Effect of the Ocean Environment on Microbial Activities*. (Colwell, R. R. & Morita, R. Y., Eds), pp. 181–201. University Park Press, Baltimore.

Bjorn, M. J., Iglewski, B. H., Ives, S. K., Sadoff, J. C. & Vasil, M. L. (1978). Effect of iron on yields of exotoxin A in cultures of *Pseudomonas aeruginosa* PA-103. *Infection and Immunity* **19,** 785–91.

Bjorn, M. J., Sokol, P. A. & Iglewski, B. H. (1979). Influence on iron yields of extracellular products in *Pseudomonas aeruginosa* cultures. *Journal of Bacteriology* **138,** 193–200.

Bryan, L. E. (1982). *Bacterial Resistance and Susceptibility to Chemotherapeutic Agents*. Cambridge University Press, Cambridge.

Davis, B. D. (1987). Mechanism of bacterial action of aminoglycosides. *Microbiological Reviews* **51,** 341–50.

Dubos, R. J. & Geiger, J. W. (1946). Preparation and properties of Shiga toxin and toxoid. *Journal of Experimental Medicine* **84,** 143–56.

Foster, J. W. & Hall, H. K. (1992). Effect of *Salmonella typhimurium* ferric uptake regulator (fur) mutations on iron and pH regulated protein synthesis. *Journal of Bacteriology* **174,** 4317–23.

Fukuda, R. & Nagasawa-Fujimori, H. (1983). Mechanism of rifampicin induction of RNA polymerase beta and beta′ subunit synthesis in *Escherichia coli*. *Journal of Biological Chemistry* **258,** 2720–28.

Goldberg, M. B., DiRita, V. J. & Calderwood, S. B. (1990). Identification of an iron-regulated virulence in *Vibrio cholerae*, using TnphoA mutagenesis. *Infection and Immunity* **58,** 55–60.

Gottlieb, S. F. (1971). Effect of hyperbaric oxygen on microorganisms. *Annual Review of Microbiology* **25,** 111–52.

Hancock, R. E. (1981). Aminoglycoside uptake and mode of action with special reference to streptomycin and gentamicin. I. Antagonists and mutants. *Journal of Antimicrobial Chemotherapy* **8,** 249–76.

Hartmann, G., Honikel, K. O., Knusel, F. & Nuesh, J. (1967). The specific inhibition of the DNA-directed RNA synthesis by rifamycin. *Biochimica et Biophysica Acta*. **145,** 843–4.

Hojyo-Tomoka, M. T., Marples, R. R. & Kligman, A. M. (1973). Pseudomonas infection in superhydrated skin. *Archives of Dermatology* **107,** 723–7.

Jones, D. M. & Davis, P. (1978). Upper respiratory tract and aural flora of saturation divers. *Journal of Clinical Pathology* **31,** 721–3.

Kenward, M. A., Alcock, S. R. & Mckay, I. C. (1984). Effect of hyperbaric oxyhelium gas on response of bacteria to antimicrobial agents in vitro. *Antimicrobial Agents and Chemotherapy* **26,** 833–6.

Lemke, P. A. & Brannon, D. R, (1972). Microbial synthesis of cephalosporin and penicillin compounds. In *Cephalosporins and Penicillins, Chemistry and Biology* (Flynn, E. H., Ed), pp. 370–437. Academic Press, New York.

Litwin, C. M. & Calderwood, S. B. (1993). Role of iron in regulation of virulence genes. *Clinical Microbiology Reviews* **6,** 137–49.

Nakamura, Y. & Yura, T. (1976). Effects of rifampicin on synthesis and functional activity of DNA-dependent RNA polymerase in *Escherichia coli*. *Molecular and General Genetics* **145,** 227–37.

Nichols, G. (1978). The effect of increased pressure on the susceptibility of some medically important microorganisms to antibiotics in common use. ARE (Physiological Laboratory). AMTE(E) Report R79 403. Gosport, UK.

Nichols, G. W., Goad, R. F. & Page, B. (1983). Skin antisepsis during steady state hyperbaric exposure and subsequent decompression. *Undersea Biomedical Research* **10,** 115–22.

Pappenheimer, A. M. & Johnson, S. J. (1936). Studies in diphtheria toxin production. I. The effect of iron and copper. *British Journal of Experimental Pathology* **17,** 335–41.

Perret, J. (1954). Iodometric assay of penicillinase. *Nature* **174,** 1012.

Sargent, M. G. (1968). Rapid fixed time assay for penicillinase. *Journal of Bacteriology* **95,** 1493.

Schlamm, N. A. (1972). Effect of elevated atmospheric pressure on antibiotic susceptibility of *Staphylococcus aureus* and *Streptococcus pyogenes*. *Antimicrobial Agents and Chemotherapy* **1,** 512.

Schlamm, N. A. & Daily, O. P. (1973). Effect of elevated atmospheric pressure on penicillin binding by *Staphylococcus aureus* and *Streptococcus pyogenes*. *Antimicrobial Agents and Chemotherapy* **3,** 147–51.

Schlamm, N. A., Perry, J. E. & Wild, J. R. (1974). Effect of helium gas at elevated pressure on iron transport and growth of *Escherichia coli*. *Journal of Bacteriology* **117,** 170–4.

Shaw, K. J., Rather, P. N., Hare, R. S. & Miller, G. H. (1993). Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiological Reviews* **57,** 138–63.

Stoebner, J. A. & Payner, S. M. (1988). Iron-regulated hemolysin production and utilization of heme and hemoglobin by *Vibrio cholerae*. *Infection and Immunity* **56,** 2891–5.

Strauss, M. B. & Dierker, R. L. (1987). Otitis externa associated with aquatic activities. *Clinical Dermatology* **5,** 103–11.

Thalman, E. D. (1974). A prophylactic programme for the prevention of otitis externa in saturation divers. U.S. Navy Experimental Diving Unit. Research Report 10-74. Washington, DC.

Victorov, A. N., Ilyin, V. K., Policarpov, N. A., Bragina, M. P., Sobolevski, V. G., Syssoev, A. D. *et al.* (1992). Microbiologic hazards for inhabitants of deep diving hyperbaric complexes. *Undersea Biomedical Research* **19,** 209–13.

Walder, D. N. (1981). Diving physiology. *Interdisciplinary Science Reviews* **6,** 67–78.

Warner, P. J., Williams, P. H., Bindereif, A. & Neilands, J. B. (1981). ColV plasmid-specified aerobactin synthesis by invasive strains of *Escherichia coli*. *Infection and Immunity* **33,** 540–5.

Wild, J. R. (1977). Induction of staphylococcal beta-lactamase in response to low concentrations of methicillin under simulated diving environments. *Canadian Journal of Microbiology* **23,** 116–121.

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