

1993). Also, it interferes with opsonophagocytosis of some strains (Smith *et al.* 1995). The position regarding serum resistance is equivocal; some papers indicate that LPS sialylation is important (Esterbrook *et al.* 1997; Kahler *et al.* 1998) and others that it is less so (Vogel *et al.* 1997; Vogel & Frosch 1999). In an outbreak of group B meningitis, an immunotype capable of LPS sialylation was associated with invasive disease and an immunotype incapable of LPS sialylation with the carrier state (Smith *et al.* 1995). The effect of lactate on meningococci has not yet been investigated.

## 5. CONCLUDING REMARKS

I hope this paper has made clear the pertinent questions about the behaviour of bacterial pathogens *in vivo*; and has indicated how they might be answered, despite difficulties in some areas, by a combination of conventional and newly devised methods.

I am indebted to J. A. Cole, M. J. Gill, N. J. Parsons C. W. Penn and E. Yates for critical reading of the manuscript.

## REFERENCES

- Abu-Kwaik, Y. & Pedersen, L. L. 1996 The use of differential display-PCR to isolate and characterize a *Legionella pneumophila* locus induced during intracellular infection of macrophages. *Mol. Microbiol.* **21**, 543–556.
- Ahmed, Z. U., Sarker, M. R. & Sack, D. A. 1990 Protection of adult rabbits and monkeys from lethal shigellosis by oral immunization with thymine requiring and sensitive mutants of *Shigella flexneri*. *Vaccine* **8**, 153–158.
- Akins, D. R., Porcella, S. F., Popova, T. G., Shevchenko, D., Buker, S. I, Li, M., Norgard, M. V. & Radolf, J. D. 1995 Evidence for *in vivo* but not *in vitro* expression of *Borrelia burgdorferi* outer surface protein F(OspF) homologue. *Mol. Microbiol.* **18**, 507–520.
- Anderson, J. D. & Smith, H. 1965 The metabolism of erythritol by *Brucella abortus*. *J. Gen. Microbiol.* **38**, 100–124.
- Anderson, J. E., Sparling, P. F. & Cornelissen, C. N. 1994 Gonococcal transferrin-binding protein 2 facilitates but is not essential for transferrin utilization. *J. Bacteriol.* **176**, 3162–3170.
- Aranda, C. M. A., Swanson, J. A., Loomis, W. P. & Miller, S. I. 1992 *Salmonella typhimurium* activates virulence gene transcription within acidified macrophage phagolysosomes. *Proc. Natl Acad. Sci. USA* **89**, 10 079–10 083.

- Arricau, N., Hermant, D., Waxin, H., Ecobichon, C., Duffey, P. S. & Popoff, M. Y. 1998 The RcsB-RcsC regulatory system of *Salmonella typhi* differentially modulates the expression of invasion proteins, flagellin and Vi antigen in response to osmolarity. *Mol. Microbiol.* **29**, 835-850.
- Bajaj, V., Lucas, R. L., Hwang, C. & Lee, C. A. 1996 Coordinate regulation of *Salmonella typhimurium* invasion genes by environmental and regulatory factors is mediated by control of *hilA* expression. *Mol. Microbiol.* **22**, 703-714.
- Bjerknes, R., Guttormsen, H., Selberg, C. O. & Wetzler, L. M. 1995 Neisserial porins inhibit human neutrophil actin polymerization, degranulation, opsonin receptor expression and phagocytosis but prime the neutrophils to increase their oxidative burst. *Infect. Immun.* **63**, 160-167.
- Bramley, J., Demarco de Hormaeche, R., Constantinidou, C., Nassif, X., Parsons, N. J., Jones, P., Cole, J. A. & Smith, H. 1996 A serum-sensitive sialyltransferase-deficient mutant of *Neisseria gonorrhoeae* defective in conversion to serum resistance by CMP-NANA or blood cell extracts. *Microb. Pathogen.* **18**, 187-195.
- Braude, A. I. & Siemienski, J. 1960 Role of bacterial urease in experimental pyelonephritis. *J. Bacteriol.* **80**, 171-179.
- Britigan, B. E., Klapper, D., Svendsen, T. & Cohen, M. S. 1988 Phagocyte-derived lactate stimulates oxygen consumption by *Neisseria gonorrhoeae*. *J. Clin. Invest.* **81**, 318-324.
- Brown, M. R. W. & Williams, P. 1985 The influence of environment on envelope properties affecting survival of bacteria in infections. *A. Rev. Microbiol.* **39**, 527-556.
- Bullen, J. J. 1981 The significance of iron in infection. *Rev. Infect. Dis.* **3**, 1127-1138.
- Burns-Keliher, L. L., Portteus, A. & Curtiss III, R. 1997 Specific detection of *Salmonella typhimurium* proteins synthesized intracellularly. *J. Bacteriol.* **179**, 3604-3612.
- Busby, S. J. W., Thomas, M. C. & Brown, N. L. 1998 *Molecular biology*. Cell biology, series H, Vol. 103. NATO ASI series. Heidelberg, Germany: Springer.
- Camilli, A. & Mekalanos, J. J. 1995 Use of recombinase gene fusions to identify *Vibrio cholerae* genes induced during infection. *Mol. Microbiol.* **18**, 671-683.
- Camilli, A., Beattie, D. T. & Mekalanos, J. J. 1994 Use of genetic recombination as a reporter of gene expression. *Proc. Natl Acad. Sci. USA* **91**, 2634-2638.
- Cannon, J. G., Johannsen, D., Hobbs, J. D., Hoffmann, H., Dempsey, J. A. F., Johnstone, D., Kayman, H. & Cohen M. S. 1998 Studies with the human challenge model of gonococcal infections; population dynamics of gonococci during experimental infection and infectivity of isogenic mutants. In *Eleventh Pathogenic Neisseria Conference* (ed. X. Nassif, M. J. Quentin-Millet & M. K. Taha), p. 48. Paris: EDK.

- Chen, L. M., Kaniga, K. & Galan, J. E. 1996 *Salmonella* spp. are cytotoxic for cultured macrophages. *Mol. Microbiol.* **21**, 1101–1115.
- Chiang, S. L. & Mekalanos, J. J. 1998 Use of signature-tagged transposon mutagenesis to identify *Vibrio cholerae* genes critical for colonization. *Mol. Microbiol.* **27**, 797–805.
- Clarke, M. A., Reece, K. A., Lodge, J., Stephen, J., Hirst, B. H. & Jepson, M. A. 1996 Invasion of murine intestinal M cells by *Salmonella typhimurium* inv mutants severely deficient for invasion of cultured cells. *Infect. Immun.* **64**, 4363–4368.
- Coates, A. R. M. & Henderson, B. 1998 Chaperonins in health and disease. *Annl's NY Acad. Sci.* **851**, 48–53.
- Cohen, M. S., Cannon, J. G., Jerse, A. E., Charniga, L. M., Isbey, S. F. & Whicker, L. G. 1994 Human experimentation with *Neisseria gonorrhoeae*: rationale, method and implication for biology of infection and vaccine development. *J. Infect. Dis.* **169**, 532–537.
- Collins, D. M. 1996 In search of tuberculosis virulence genes. *Trends Microbiol.* **4**, 426–430.
- Conner, C. P., Heithoff, D. M. & Mahan, M. J. 1998 *In vivo* gene expression: contributions to infection, virulence, and pathogenesis. *Curr. Top. Microbiol. Immunol.* **225**, 1–12.
- Contag, C. H., Contag, P. R., Mullins, J. I., Spilman, S. D., Stevenson, D. K. & Benaron, D. A. 1995 Photonic detection of bacterial pathogens in living hosts. *Mol. Microbiol.* **18**, 593–603.
- Cornelis, G. R. 1998 The *Yersinia* deadly kiss. *J. Bacteriol.* **180**, 5495–5504.
- Cornelis, G. R., Boland, A., Boyd, A. P., Geuijen, C., Iriate, M., Neyt, C., Sory, M. P. & Stainier, I. 1998 The virulence plasmid of *Yersinia*; an antihost genome. *Microbiol. Mol. Biol. Rev.* **62**, 1315–1352.
- Cornelissen, C. N., Biswas, G. D., Tsai, J., Parachuri, D. K., Thompson, S. A. & Sparling, P. F. 1992 Gonococcal transferrin-binding protein 1 is required for transferrin utilization and is homologous to Ton-B-dependent outer membrane receptors. *J. Bacteriol.* **174**, 5788–5797.
- Cornelissen, C. N., Kelly, M., Hobbs, M. M., Anderson, J. F., Cannon, J. G., Cohen, M. S. & Sparling, P. F. 1997 The transferrin receptor expressed by gonococcal strain FA1090 is required for experimental infection of human male volunteers. *Mol. Microbiol.* **27**, 611–616.
- Coulter, S. N., Schwan, W. R., Ng, E. Y. W., Langhorne, M. H., Ritchie, H. D., Westbrook-Wadman, S., Hufnagle, W. O., Folger, K. R., Bayer, A. S. & Stover, C. K. 1998 *Staphylococcus aureus* genetic loci impacting growth and survival in multiple infection environments. *Mol. Microbiol.* **30**, 393–404.
- Crooke, H., Griffiss, J. M., John, C. M., Lissenden, S., Bramley, J., Regan, T., Smith, H. & Cole, J. A. 1998 Characterization of a sialyltransferase-deficient mutant of *Neisseria gonorrhoeae* strain F62; instability of transposon Tn1595 $\Delta$ 3 in gonococci and evidence that multiple genetic loci are essential for lipooligosaccharide sialylation. *Microb. Pathogen.* **25**, 237–252.

- Culham, D. E., Dalgado, C., Gyles, C. L., Mamelah, D., MacLellan, S. & Wood, J. M. 1998 Osmoregulatory transporter ProP influences colonization of the urinary tract by *Escherichia coli*. *Microbiology* **144**, 91–102.
- Curtiss III, R., Kelly, S. M., Gulig, P. A., Gentry-Weeks, C. R. & Galan, J. E. 1988 Avirulent salmonellae expressing virulence antigens from other pathogens for use as orally administered vaccines. In *Virulence mechanisms of bacterial pathogens* (ed. J. A. Roth), pp. 311–328. Washington, DC: American Society for Microbiology.
- De Saizieu, A., Certa, U., Warrington, J., Gray, C., Keck, W. & Mous, J. 1998 Bacterial transcript imagery by hybridization of total RNA to oligonucleotide arrays. *Nat. Biotech.* **16**, 45–48.
- Dehio, C., Gray-Owen, S. D. & Meyer, T. F. 1998 The role of Opa proteins in interactions with host cells. *Trends Microbiol.* **6**, 489–495.
- Demarco de Hormaeche, R., Mehlert, A., Young, D. B. & Hormaeche, C. E. 1991 Antigenic homology between the 65 kDa heat shock proteins of *Mycobacterium tuberculosis*, GroEl of *E. coli* and proteins of *Neisseria gonorrhoeae* expressed during infection. In *Neisseria 1990* (ed. M. Achtman, P. Kohl, C. Marchal, G. Morelli, A. Seiler & B. Thiesen), pp. 199–203. New York: Walter de Gruyter.
- Esterbrook, M. E., Griffiss, J. M. & Jarvis, G. A. 1997 Sialylation of *Neisseria meningitidis* lipooligosaccharide inhibits serum bactericidal activity by masking lacto-N-tetraose. *Infect. Immun.* **65**, 4436–4444.
- Falkow, S. 1988 Molecular Koch's postulates applied to microbial pathogenicity. *Rev. Infect. Dis.* **10**, S274–S276.
- Fields, P. I., Swanson, R. V., Haidaris, C. G. & Heffron, F. 1986 Mutants of *Salmonella typhimurium* that cannot survive within macrophages are avirulent. *Proc. Natl Acad. Sci. USA* **83**, 5189–5193.
- Finlay, B. B. & Falkow, S. 1997 Common themes in microbial pathogenicity revisited. *Microbiol. Mol. Biol. Rev.* **61**, 136–169.
- Galan, J. E. 1996 Molecular genetic bases of salmonella entry into host cells. *Mol. Microbiol.* **20**, 263–271.
- Gao, L., Parsons, N. J., Curry, A., Cole, J. A. & Smith, H. 1998 Lactate causes changes in gonococci including increased lipopolysaccharide synthesis during short-term incubation in media containing glucose. *FEMS Microbiol. Lett.* **169**, 309–316.
- Garcia-del Portillo, F., Foster, J. W., Maguire, M. E. & Finlay, B. B. 1992 Characterization of the microenvironment of *Salmonella typhimurium* containing vacuoles within MDCK epithelial cells. *Mol. Microbiol.* **6**, 3289–3297.
- Garcia Vescovi, E., Soncini, F. C. & Groisman, E. A. 1994 The role of the PhoP/PhoQ regulon in *Salmonella* virulence. *Res. Microbiol.* **145**, 473–480.
- Garcia Vescovi, E., Soncini, F. C. & Groisman, E. A. 1996 Mg<sup>2+</sup> as an extracellular signal: environmental regulation of *Salmonellae* virulence. *Cell* **84**, 165–174.

- Gilbert, M., Watson, D. C., Cunningham, A.-M., Jennings, M. P., Young, N. M. & Wakarchuk, W. W. 1996 Cloning of the lipooligosaccharide  $\alpha$ -2,3-sialyltransferase from the bacterial pathogens *Neisseria meningitidis* and *Neisseria gonorrhoeae*. *J. Biol. Chem.* **271**, 28 271–28 276.
- Gill, M. J., McQuillen, D. P., Van Putten, J. P. M., Wetzler, L. M., Bramley, J., Crooke, H., Parsons, N. J., Cole, J. A. & Smith, H. 1996 Functional characterization of a sialyltransferase deficient mutant of *Neisseria gonorrhoeae*. *Infect. Immun.* **64**, 3374–3378.
- Gillaspay, A. F., Hickmon, S. G., Skinner, R. A., Thomas, T. R., Nelson, C. L. & Smeltzer, M. S. 1995 Role of the accessory gene regulator (*agr*) in pathogenesis of staphylococcal osteomyelitis. *Infect. Immun.* **63**, 3373–3380.
- Gotschlich, E. M., Seiff, M. E., Blake, M. S. & Koomey, M. 1987 Porin protein of *Neisseria gonorrhoeae*: cloning and gene structure. *Proc. Natl Acad. Sci. USA* **84**, 8135–8139.
- Guangyong, J., Beavis, R. C. & Novick, R. P. 1995 Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. *Proc. Natl Acad. Sci. USA* **92**, 12 055–12 059.
- Gulig, P. A. & Doyle, T. J. 1993 The *Salmonella typhimurium* virulence plasmid increases the growth rate of salmonellae in mice. *Infect. Immun.* **61**, 504–511.
- Guo, L., Lim, K. B., Gunn, J. S., Bainbridge, B., Darveau, R. P., Hackett, M. & Miller, S. I. 1997 Regulation of lipid A modification by *Salmonella typhimurium* virulence genes *phoP-phoQ*. *Science* **276**, 250–253.
- Haas, M., Lindner, B., Seydel, U. & Levy, L. 1993 Comparison of the intrabacterial  $\text{Na}^+\text{K}^+$  ratio and multiplication in the mouse foot pad as measures of the proportion of viable *Mycobacterium leprae*. *Int. J. Antimicrobiol. Agents* **2**, 117–128.
- Halle, F. & Meyer, J. M. 1992 Iron release from ferrisiderophores. A multistep mechanism involving a NADH/FMN oxidoreductase and chemical reduction by FMNH. *Eur. J. Biochem.* **209**, 621–627.
- Heithoff, D. M., Conner, C. P., Hanna, P. C., Julio, S. M., Henschel, U. & Mahan, M. J. 1997 Bacterial infection assessed by *in vivo* gene expression. *Proc. Natl Acad. Sci. USA* **94**, 934–939.
- Heithoff, D. M., Conner, C. P., Henschel, U., Govantes, F., Hanna, P. C. & Mahan, M. J. 1999 Coordinate intracellular expression of *Salmonella* genes induced during infection *in vivo*. *J. Bacteriol.* **181**, 799–807.
- Hensel, M., Shea, J. E., Gleeson, C., Jones, M. D., Dalton, E. & Holden, D. W. 1995 Simultaneous identification of bacterial virulence genes by negative selection. *Science* **269**, 400–403.
- Hensel, M., Shea, J. E., Waterman, S. R., Mundy, R., Nikolaus, T., Banks, G., Vazquez-Torres, A., Gleeson, C., Fang, F. C. & Holden, D. W. 1998 Genes encoding putative effector proteins of the type III secretion system of *Salmonella* pathogenicity island 2 are required for bacterial virulence and proliferation in macrophages. *Mol. Microbiol.* **30**, 163–174.

- Herbert, S., Werlitzsch, D., Dussy, B., Nontonnier, A., Fournier, J. M., Belton, G., Dolhoff, A. & Daring, G. 1997 Regulation of *Staphylococcus aureus* capsular polysaccharide type 5: CO<sub>2</sub> inhibition *in vitro* and *in vivo*. *J. Infect. Dis.* **176**, 431–438.
- Herrington, D. A., Hall, R. H., Lasonsky, G., Mekalanos, J. J., Taylor, R. K. & Levine, M. A. 1988 Toxin, toxin-coregulated pili and the *toxR* regulon are essential for *Vibrio cholerae* pathogenesis in humans. *J. Exp. Med.* **168**, 1487–1492.
- Hooke, A. M., Sordelli, D. O., Cerquetti, H. C. & Vogt, A. J. 1985 Quantitative determination of bacterial replication *in vivo*. *Infect. Immun.* **49**, 424–427.
- Hormaeche, C. E. 1980 The *in vivo* division and death rate of *Salmonella typhimurium* in the spleens of naturally resistant and susceptible mice measured by the superinfecting phage technique of Meynell. *Immunology* **41**, 973–979.
- Hueck, C. J. 1998 Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol. Mol. Biol. Rev.* **62**, 379–433.
- Huynen, M. A., Dandekar, T. & Bork, P. 1999 Variation and evolution of the citric-acid cycle: a genomic perspective. *Trends Microbiol.* **7**, 281–291.
- Jepson, M. A. & Clark, M. A. 1998 Studying M cells and their role in infection. *Trends Microbiol.* **6**, 359–365.
- Jerse, A. E., Cohen, M. S., Drown, P. M., Whicker, L. G., Isbey, S. F., Seifert, H. S. & Cannon, J. G. 1994 Multiple gonococcal opacity proteins are expressed during experimental urethral infection in the male. *J. Exp. Med.* **179**, 911–920.
- Jones, B. D., Ghorri, N. & Falkow, S. 1994 *Salmonella typhimurium* initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. *J. Exp. Med.* **180**, 15–23.
- Kahler, C. M., Martin, L. E., Shih, G. C., Rahman, M. M., Carlson, R. W. & Stephens, D. S. 1998 The ( $\alpha$ 2-8)linked polysialic capsule and lipopolysaccharide structure both contribute to ability of serogroup N *Neisseria meningitidis* to resist the bactericidal activity of normal human serum. *Infect. Immun.* **66**, 5939–5945.
- Karnell, A., Cam, P. D., Verna, N. & Lindberg, A. A. 1993 *AroD* deletion attenuates *Shigella flexneri* strain 2457T and makes it a safe and efficacious oral vaccine in monkeys. *Vaccine* **8**, 830–836.
- Keppie, J., Williams, A. E., Witt, K. & Smith, H. 1965 The role of erythritol in the tissue localization of the brucellae. *Br. J. Exp. Pathol.* **46**, 104–108.
- Kolter, R. 1999 Evolution of microbial diversity during prolonged starvation. *Proc. Natl Acad. Sci. USA* **96**, 4023–4027.
- Kolter, R., Siegele, D. A. & Tormo, A. 1993 The stationary phase of the bacterial life cycle. *A. Rev. Microbiol.* **47**, 855–874.

- Latifi, A., Foglino, M., Tanaka, K., Williams, P. & Lazdunski, A. 1996 A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators LasR and RhlR (VsmR) to the stationary phase sigma factor RpoS. *Mol. Microbiol.* **21**, 1137–1146.
- Leclerc, G. J., Tartera, C. & Metcalf, E. S. 1998 Environmental regulation of *Salmonella typhi* invasion-defective mutants. *Infect. Immun.* **66**, 682–691.
- Lentner, C. 1981 Units of measurement, body fluids, composition of the body, nutrition. In *Geigy scientific tables*, Vol. 1. Basle, Switzerland: Ciba Geigy.
- Lentner, C. 1984 Physical chemistry, composition of the blood, haematology, sonatometric data. In *Geigy scientific tables*, Vol. 3. Basle, Switzerland: Ciba Geigy.
- Leung, K. Y. & Finlay, B. B. 1991 Intracellular replication is essential for virulence of *Salmonella typhimurium*. *Proc. Natl Acad. Sci. USA* **88**, 11 470–11 474.
- Levine, M. M., Herrington, D., Murphy, J. R., Morris, J. G., Losonsky, G., Tall, B., Lindberg, A. A., Svenson, S., Bagar, S., Edwards, M. F. & Stocker, B. 1987 Safety, infectivity, immunogenicity and *in vivo* stability of two attenuated auxotrophic mutant strains of *Salmonella typhi* 541Ty and 543Ty used as oral vaccines for man. *J. Clin. Invest.* **79**, 888–902.
- Lockhardt, D. J., Dong, H., Byrn, M. C., Follettie, M. T., Gallo, M. W., Chie, M. S., Mittmann, M., Wang, C., Kobayashi, M., Horton, H. & Brown, E. L. 1996 Expression monitoring by hybridisation to high density oligonucleotide arrays. *Nat. Biotech.* **14**, 1675–1680.
- Lowe, A. M., Beattie, D. T. & Deresiewicz, R. C. 1998 Identification of novel staphylococcal virulence genes by *in vivo* expression technology. *Mol. Microbiol.* **27**, 967–976.
- McCormick, B. A., Stocker, B. A. D., Laux, D. C. & Cohen, P. S. 1988 Roles of motility, chemotaxis and penetration through and growth in intestinal mucus in the ability of an avirulent strain of *Salmonella typhimurium* to colonize the large intestine of streptomycin treated mice. *Infect. Immun.* **56**, 2209–2217.
- MacLaren, D. M. 1968 The significance of urease in proteus pyelonephritis. Bacteriological study. *J. Pathol. Bact.* **96**, 45–51.
- McNeil, G. & Virgi, M. 1997 Phenotypic variants of meningococci and their potential in phagocytic interactions: the influence of opacity proteins, pili, PilC and surface sialic acids. *Microb. Pathogen.* **22**, 295–304.
- Mahan, M. J., Slauch, J. M. & Mekalanos, J. J. 1993 Selection of bacterial virulence genes that are specifically induced in the host tissues. *Science* **259**, 686–688.
- Mahan, M. J., Slauch, J. M., Hanna, P. C., Camilli, A., Tobias, J. W., Waldor, M. K. & Mekalanos, J. J. 1994 Selection for bacterial genes that are specifically induced in host tissues: the hunt for virulence factors. *Infect. Agents Dis.* **2**, 263–268.

- Mahan, M. J., Tobias, J. W., Slauch, J. M., Hanna, P. C., Collier, R. J. & Mekalanos, J. J. 1995 Antibiotic-based selection for bacterial genes that are specifically induced during infection of a host. *Proc. Natl Acad. Sci. USA* **92**, 669–673.
- Mantle, M. & Rombough, C. 1993 Growth in and breakdown of purified rabbit small intestinal mucus by *Yersinia enterocolitica*. *Infect. Immun.* **61**, 4131–4138.
- Maw, J. & Meynell, G. G. 1968 The true division and death rates of *Salmonella typhimurium* in the mouse spleen determined with superinfecting phage P22. *Br. J. Exp. Path.* **49**, 597–613.
- Mei, J. M., Nourbakhsh, F., Ford, C. W. & Holden, D. W. 1997 Identification of *Staphylococcus aureus* genes in a murine model of bacteraemia using signature-tagged mutagenesis. *Mol. Microbiol.* **26**, 399–407.
- Mikulskis, A. V., Delor, I., Thi, V. H. & Cornelis, G. R. 1994 Regulation of the *Yersinia enterocolitica* enterotoxin Yst gene. Influence of growth phase, temperature, osmolarity, pH and bacterial host factors. *Mol. Microbiol.* **14**, 905–915.
- Miller, J. F., Kukral, A. M. & Mekalanos, J. J. 1989 A two-component regulatory system (*phoP/phoQ*) controls *Salmonella typhimurium* virulence. *Proc. Natl Acad. Sci. USA* **86**, 5054–5058.
- Morgan, A. J. 1985 *X-ray microanalysis: electron microscopy for biologists*. Oxford University Press.
- Nauman, M., Hanski, C. & Reichen, E. O. 1991 Expression *in vivo* of additional plasmid-mediated proteins during intestinal infection with *Yersinia enterocolitica* serotype O8. *J. Med. Microbiol.* **35**, 257–261.
- O'Callaghan, D., Maskell, D., Liew, F. Y., Easmon, C. S. F. & Dougan, D. 1988 Characterization of aromatic- and purine-dependent *Salmonella typhimurium*; attenuated persistence, and ability to induce protective immunity in BALB/c mice. *Infect. Immun.* **56**, 419–423.
- Parsons, N. J., Boons, G. J., Ashton, P. R., Redfern, P. D., Quirk, P., Gao, Y., Constantinidou, C., Patel, J., Bramley, J., Cole, J. A. & Smith, H. 1996a Lactic acid is the factor in blood cell extracts which enhances the ability of CMP-NANA to sialylate gonococcal lipopolysaccharide and induce serum resistance. *Microb. Pathogen.* **20**, 87–100.
- Parsons, N. J., Emond, J. P., Goldner, M., Bramley, J., Crooke, H., Cole, J. A. & Smith, H. 1996b Lactate enhancement of sialylation of gonococcal lipopolysaccharide and induction of serum resistance by CMP-NANA is not due to direct activation of the sialyltransferase: metabolic events are involved. *Microb. Pathogen.* **21**, 193–204.
- Pascopella, L., Collins, F. M., Martin, J. M., Lee, M. H., Hatfull, G. F., Stover, C. K., Bloom, B. R. & Jacobs, W. R. 1994 Use of *in vivo* complementation in *Mycobacterium tuberculosis* to identify a genome fragment associated with virulence. *Infect. Immun.* **62**, 1313–1319.

- Pepe, J. C., Badger, J. L. & Miller, V. L. 1994 Growth phase and low pH affect the thermal regulation of the *Yersinia enterocolitica* inv. gene. *Mol. Microbiol.* **11**, 123–135.
- Perin, F., Laurence, D., Savary, I., Bernard, S. & Le Pape, A. 1997 Radioactive technetium-99m labelling of *Salmonella abortusovis* for assessment of bacterial dissemination in sheep by *in vivo* imaging. *Vet. Microbiol.* **51**, 171–180.
- Pesci, E. C. & Iglewski, B. H. 1997 The chain of command in *Pseudomonas* quorum sensing. *Trends Microbiol.* **5**, 133–135.
- Petterson, J., Nordfelth, R., Dubinina, E., Bergman, T., Gustafsson, M., Magnusson, K. E. & Wolf-Watz, H. 1996 Modulation of virulence factor expression by pathogen target cell contact. *Science* **273**, 1231–1233.
- Plum, G. & Clark-Curtiss, J. E. 1994 Induction of *Mycobacterium avium* gene expression following phagocytosis by human macrophages. *Infect. Immun.* **62**, 476–483.
- Pollack, C., Straley, S. C. & Klempner, M. S. 1986 Probing the phagolysosome environment of human phagocytes with a Ca<sup>2+</sup> responsive operon fusion in *Yersinia pestis*. *Nature* **332**, 834–836.
- Preston, M. J., Seed, P. C., Toder, D. S., Iglewski, B. H., Ohman, D. E., Gustin, J. K., Goldberg, J. B. & Pier, G. P. 1997 Contributions of proteases and LasR to virulence of *Pseudomonas aeruginosa* during corneal infection. *Infect. Immun.* **65**, 3086–3090.
- Ramsay, G. 1998 DNA chips: state of the art. *Nat. Biotech.* **16**, 40–44.
- Regan, T., Watts, A., Smith, H. & Cole, J. A. 1999 Regulation of the lipopolysaccharide-specific sialyltransferase activity of gonococci by growth state of the bacteria, but not by carbon source, catabolite repression or oxygen supply. *Anton von Leeuwenhoek* **75**, 369–379.
- Richter-Dahlfors, A., Buchan, A. M. J. & Finlay B. B. 1997 Murine salmonellosis studied by confocal microscopy: *Salmonella typhimurium* resides intracellularly inside macrophages and exerts a cytotoxic effect on phagocytes *in vivo*. *J. Exp. Med.* **186**, 569–580.
- Russo, T. A., Jadush, S. T., Brown, J. J. & Johnson, J. R. 1996 Identification of two previously unrecognised genes (*guaA* and *argC*) important for uropathogenesis. *Mol. Microbiol.* **22**, 217–228.
- Sansonetti, P. J., Nhieu, G. T. V. & Egile, C. 1999 Rupture of the intestinal epithelial barrier and mucosal invasion by *Shigella flexneri*. *Clin. Infect. Dis.* **28**, 466–475.
- Schena, M., Shalon, D., Heller, R., Chai, A., Brown, P. O. & Davis, R. W. 1996 Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. *Proc. Natl Acad. Sci. USA* **93**, 10 614–10 619.
- Schneider, H., Griffiss, J. M., Boslego, J. W., Hitchcock, P. J., Zahos, K. M. & Apicella, M. A. 1991 Expression of paragloboside-like lipooligosaccharides may be a necessary component of gonococcal pathogenesis in men. *J. Exp. Med.* **174**, 1601–1605.

- Schneider, H., Cross, A. S., Kuschner, R. R., Taylor, D. N., Sadoff, J. C., Boslego, J. W. & Deal, C. D. 1995 Experimental human gonococcal urethritis: 250 *Neisseria gonorrhoeae* MSIIImkC are infective. *J. Infect. Dis.* **172**, 180–185.
- Schneider, H., Schmidt, K. A., Skillman, D. R., Van De Verg, L., Warren, R. L., Wylie, H. J., Sadoff, J. C., Deal, C. D. & Cross, A. S. 1996 Sialylation lessens the infectivity of *Neisseria gonorrhoeae* MSIIImkC. *J. Infect. Dis.* **173**, 1422–1427.
- Schwan, W. R., Coulter, S. N., Ng, E. Y. W., Langhorne, M. H., Ritchie, H. D., Brody, L. L., Westbrook-Wadman, S., Bayer, A. S., Folger, K. R. & Stover, C. K. 1998 Identification and characterization of the PutP proline permease that contributes to *in vivo* survival of *Staphylococcus aureus* in animal models. *Infect. Immun.* **66**, 567–572.
- Seydel, U., Haas, M., Rietschel, E. T. & Lindner, B. 1992 Laser probe mass spectrometry of individual bacteria organisms and of isolated bacterial compounds; a tool in microbiology. *J. Microbiol. Meth.* **15**, 167–181.
- Shea, J. E., Hensel, M., Gleeson, C. & Holden, D. W. 1996 Identification of a virulence locus encoding a second type III secretion system in *Salmonella typhimurium*. *Proc. Natl Acad. Sci. USA* **93**, 2593–2597.
- Skorupski, K. & Taylor, R. K. 1997 Control of the ToxR virulence regulon in *Vibrio cholerae* by environmental stimuli. *Mol. Microbiol.* **25**, 1003–1009.
- Smith, H. 1990 Pathogenicity and the microbe *in vivo*. *J. Gen. Microbiol.* **136**, 377–393.
- Smith, H. 1995 The revival of interest in mechanisms of bacterial pathogenicity. *Biol. Rev.* **70**, 277–316.
- Smith, H. 1996 What happens *in vivo* to bacterial pathogens? *Annls NY Acad. Sci.* **797**, 77–92.
- Smith, H., Anderson, J. D., Keppie, J., Kent, P. W. & Timmis, C. M. 1965 The inhibition of the growth of *Brucellae in vitro* and *in vivo* by analogues of erythritol. *J. Gen. Microbiol.* **38**, 101–108.
- Smith, H., Williams, A. E., Pearce, J. H., Keppie, J., Harris-Smith, P. W., Fitzgeorge, R. B. & Witt, K. 1962 Foetal erythritol: a cause of the tissue localization of *Brucella abortus* in bovine contagious abortion. *Nature* **193**, 47–49.
- Smith, H., Cole, J. A. & Parsons, N. J. 1995 Sialylation of neisserial lipopolysaccharide: a major influence on pathogenicity. *Microb. Pathogen.* **19**, 365–377.
- Sordelli, D. O., Cerquetti, M. C. & Hooke, A. M. 1985 Replication rate of *Pseudomonas aeruginosa* in the murine lung. *Infect. Immun.* **50**, 388–391.
- Spencer, A. J., Osbourne, M. P., Haddon, S. J., Collins, J., Starkey, W. G., Candy, D. C. A. & Stephen, J. 1990 X-ray microanalysis of rotavirus-infected mouse intestine: a new concept of diarrhoeal secretion. *J. Pediatr. Gastroenterol. Nutr.* **10**, 516–529.
- Storey, D. G., Ujack, E. E., Rabin, H. R. & Mitchell, I. 1998 *Pseudomonas aeruginosa lasR* transcription correlates with the transcription of *lasA*, *lasB*, and

- toxA* in chronic lung infections associated with cystic fibrosis. *Infect. Immun.* **66**, 2521–2528.
- Strauss, E. J. & Falkow, S. 1997 Microbial pathogenesis, genomes and beyond. *Science* **276**, 701–712.
- Sud, I. J. & Feingold, D. S. 1975 Phospholipids and fatty acids of *Neisseria gonorrhoeae*. *J. Bacteriol.* **124**, 713–717.
- Suk, K., Das, S., Sun, W., Jwang, B., Barthold, S. W., Flavell, R. A. & Fikrig, E. 1995 *Borrelia burgdorferi* genes selectively expressing in the infected host. *Proc. Natl Acad. Sci. USA* **92**, 4269–4273.
- Tang, C. & Holden, D. W. 1999 Pathogen virulence genes-implications for vaccines and drug therapy. *Br. Med. Bull.* **55**, 387–400.
- Tang, H. B., DiMango, E., Bryan, R., Gambello, M., Iglewski, B. H., Goldberg, J. P. & Prince, A. 1996 Contribution of specific *Pseudomonas aeruginosa* virulence factors to pathogenesis of pneumonia in a neonatal mouse model of infection. *Infect. Immun.* **64**, 37–43.
- Valdivia, R. H. & Falkow, S. 1996 Bacterial genetics by flow cytometry: rapid isolation of *Salmonella typhimurium* acid inducible promoters by differential fluorescence induction. *Mol. Microbiol.* **23**, 367–378.
- Valdivia, R. H. & Falkow, S. 1997a Fluorescence-based isolation of bacterial genes expressed within host cells. *Science* **277**, 2007–2011.
- Valdivia, R. H. & Falkow, S. 1997b Probing bacterial gene expression within host cells. *Trends Microbiol.* **5**, 360–363.
- Virgi, M., Makepeace, K., Ferguson, D. J. P., Acktman, M. & Moxon, E. R. 1993 Meningococcal Opa and Opc proteins; their role in colonization and invasion of epithelial and endothelial cells. *Mol. Microbiol.* **10**, 499–510.
- Vogel, U. & Frosch, M. 1999 Mechanisms of neisserial serum resistance. *Mol. Microbiol.* **32**, 1133–1139.
- Vogel, U., Claus, H., Heinze, G. & Frosch, M. 1997 Functional characterization of an isogenic meningococcal  $\alpha$ 2-3sialyltransferase mutant: the role of lipopolysaccharide sialylation for serum resistance in serogroup B meningococci. *Med. Microbiol. Immunol.* **186**, 159–166.
- Wachtel, M. R. & Miller, V. L. 1995 *In vitro* and *in vivo* characterization of an ail mutant of *Yersinia enterocolitica*. *Infect. Immun.* **63**, 2541–2548.
- Wallich, R., Brenner, C., Kramer, M. D. & Simon, M. M. 1995 Molecular cloning and immunological characterization of a novel linear-plasmid-encoded gene *pG* of *Borrelia burgdorferi* expressed only *in vivo*. *Infect. Immun.* **63**, 3327–3335.
- Wang, J., Lory, S., Ramphal, R. & Jin, S. 1996a Isolation and characterization of *Pseudomonas aeruginosa* genes inducible by respiratory mucus derived from cystic fibrosis patients. *Mol. Microbiol.* **22**, 1005–1012.
- Wang, J., Mushagian, A., Lory, S. & Jin, S. 1996b Large scale isolation of candidate virulence genes of *Pseudomonas aeruginosa* by *in vivo* selection. *Proc. Natl Acad. Sci. USA* **93**, 10 434–10 439.

- Weinberg, E. D. 1995 Acquisition of iron and other nutrients *in vivo*. In *Virulence mechanisms of bacterial pathogens*, 2nd edn (ed. J. A. Roth, C. A. Bolin, R. A. Brogden, F. C. Minion and M. J. Wannemuehler), pp. 79–93. Washington, DC: American Society for Microbiology.
- Williams, A. E., Keppie, J. & Smith, H. 1964 The relation of erythritol usage to virulence in *Brucellas*. *J. Gen. Microbiol.* **37**, 265–292.
- Winson, M. K. (and 12 others) 1995 Multiple N-acyl-L-homoserinelactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA* **92**, 9427–9431.
- Yates, E., Gao, L., Parsons, N. J., Cole, J. A. & Smith, H. 1999 When lactate is used by gonococci growing in a medium containing glucose as occurs *in vivo*, its carbon is incorporated into lipid, LPS, GroEl and porin 1B. In *Abstracts of a Royal Society Meeting on the 'Activities of bacterial pathogens in vivo'*. Reading, UK: Society for General Microbiology.
- Young, G. M. & Miller, V. L. 1997 Identification of novel chromosome loci affecting *Yersinia enterocolitica* pathogenesis. *Mol. Microbiol.* **25**, 319–328.