

the brain of the abnormal form of the prion protein.

Importantly, a few of the atypical cases are in 'resistant' sheep. Thus, Buschmann and co-workers³ reported two atypical cases of scrapie in sheep of the ARR/ARR type in Germany, and Orge and co-workers⁴ describe a third in Portugal. Three further cases have been reported in France⁵. These discoveries confirm, under natural conditions, a finding made in sheep experimentally inoculated with BSE: ARR/ARR sheep are not, after all, totally resistant to TSEs¹⁰.

These atypical scrapie cases show some similarity to a new strain of scrapie, first detected in Norway in 1998 (ref. 11). Moum and co-workers⁶ have now genetically tested all 38 Norwegian Nor98 cases, as well as the unaffected sheep in the flocks from which they came, allowing the relative susceptibility or resistance of each genetic type to be estimated. The results transform our understanding of the relationship between PrP haplotype and scrapie. Sheep that are the most susceptible to conventional scrapie appear to be unaffected by Nor98 (Fig. 1). Conversely, susceptibility to Nor98 is linked to the AHQ haplotype, which is generally associated with resistance (or, at most, low susceptibility) to conventional scrapie. Nor98 is also being identified over a wider area in Europe. It has been reported in Norway, Sweden, Finland and, in November 2004, in Ireland¹² and Belgium¹³.

Encouragingly, there have been no cases among ARR/ARR sheep, and so it is possible that national flocks of this genetic type may be resistant to both conventional and Nor98 scrapie. A warning shot has, however, been fired. The existence of Nor98 shows that sheep largely resistant to 'known' strains of scrapie might be highly susceptible to a novel strain identified in the future; and the discovery of atypical infections in ARR/ARR sheep presses the point that we cannot exclude these sheep from this risk. Larger epidemics of scrapie tend to be associated with flocks that have high proportions of sheep susceptible to the local scrapie strain. Accordingly, as the ARR haplotype increasingly dominates European sheep populations, there is a danger that epidemics of a strain able to attack this haplotype could be larger than those the region has experienced in the past.

One final point: the existence of the VRQ haplotype has been enigmatic, as it is not known to confer any benefits, but is often fatal for sheep exposed to conventional scrapie. Why has it not been eliminated from sheep populations by natural selection? The findings of Moum *et al.*⁶ raise the possibility that this haplotype exists today because it conferred resistance to past strains. If so, this is a strong argument for the preservation of haplotype diversity in sheep populations, and the long-term control of scrapie

in Europe may be better achieved by the US approach: eliminating infection within infected flocks and their contacts, while preserving haplotype diversity at a national scale.

Matthew Baylis and K. Marie McIntyre are at the Institute for Animal Health, Compton, Newbury, Berkshire RG20 7NN, UK.

e-mail: matthew.baylis@bbsrc.ac.uk

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Cell biology

Iron thievery

Jonathan Barasch and Kiyoshi Mori

Bacteria have many ways of stealing iron from the organisms they infect. But this thievery is not one-sided, and a newly discovered device in the mammalian tool kit does a good job of keeping bacteria in check.

A fierce battle rages between man and microbe over iron. The breakthrough reported by Flo and colleagues¹ on page 917 of this issue exemplifies the lengths to which microorganisms must go to obtain iron — and the remarkable ingenuity of our own natural countermeasures for denying them this essential metal. So far, humans are winning, and we're doing it with a member of an obscure family of proteins called the lipocalins.

Iron is a problem for all that live by it. This is because the ferrous ion, although soluble in biological fluids, generates toxic hydroxyl radicals in the presence of oxygen. The oxidized (ferric) form is better behaved, but it is insoluble in water at neutral pH (ref. 2), producing ferrihydroxide precipitates; ferriphosphate precipitates also form with the biologically abundant phosphate ion. It is somewhat paradoxical that these iron ions are so inaccessible, given that iron is the key catalytic site of many of the enzymes and gas-transporting proteins in cells.

To solve the problem of taking up iron into cells, organisms have developed numerous iron-solubilization technologies. In mammals, birds, fish and amphibians, a highly conserved protein called transferrin is needed to move the major load of extracellular iron into cells. Transferrin binds extracellular iron with high affinity (10^{-20} M), docks at transferrin receptors on the cell membrane, and is taken up into cells by means of the invagination of sections of this membrane. The iron is then unloaded from transferrin into specialized intracellular compartments, from where it can be transferred to the cytoplasm.

In bacteria and fungi, meanwhile, other iron-capturing tools are brought into play.

For instance, these organisms can secrete and then reclaim a bewildering array of iron-avid peptides and ester derivatives, known collectively as siderophores³. These are classified as phenolates–catecholates or hydroxamates, or mixtures of the two forms. Enterochelin, a major catecholate siderophore, chelates iron with extraordinarily high affinity (10^{-49} M)⁴, docks at unique receptors — the Fep proteins — on the bacterial surface, and is destroyed by esterases after being internalized.

So what happens when bacteria grow in a host that also covets iron (mammalian blood serum has just 10^{-26} M free iron⁵), or when two microorganisms compete for the same source of metal? Essentially, thievery reigns. For instance, when in competition with fungi, bacteria can hijack iron that is targeted to the fungi by producing receptors for fungal siderophores that the bacteria themselves do not make. When in competition with animal hosts, bacteria can expropriate transferrin (or the related lactoferrin) wholesale, or simply remove its iron by using siderophores. Mycobacteria use the siderophore carboxymycobactin⁶ to strip iron from mammalian ferritin, another iron-binding protein. And fungi of the genus *Rhizopus*⁷ have been reported to capture an iron-chelating medicine, deferoxamine, allowing these organisms to multiply to levels that are disastrous for the host. Perhaps not surprisingly, giving iron to patients⁸ (and, as Flo *et al.* show¹, to mice) worsens the outcome of bacterial and mycobacterial infections.

But the hosts have a few tricks of their own. If they hoard iron, they can control the microbial population. One way of doing this is to reduce the amounts of transferrin. Iron recycling from macrophage cells (a type of immune cell) can also be shut down. And



100 YEARS AGO

Earthquakes. By Clarence Edward Dutton. Major Dutton's work belongs to another category, and rather than telling us what earthquakes do, his main object has been to tell us what they are... Everything is discussed with a minimum of mathematics from a strictly scientific standpoint, whilst that which is sensational has properly been most carefully put under taboo. A justification for the exclusion of what is of practical importance, which gives not only to the man in the street but to Governments some inkling as to the use of earthquakes, is not so apparent. It is extremely likely that a Prime Minister may not care a twopenny-bit whether the inside of the world on which he lives is red hot stone or cold, while he might be extremely interested to know that seismograms may afford a satisfactory explanation for the interruption of his cablegrams. The importance of earthquake writings to communities who have been alarmed by accounts of disasters in foreign countries is self-evident, while it would at least be consoling to those who were suddenly cut off from the outer world by the failure of their cables to learn whether such failures were the result of an operation of war or of nature.

From *Nature* 15 December 1904.

50 YEARS AGO

The structure of fibrous proteins has long been a subject of controversy. X-ray and electron microscope evidence has accumulated which suggests that single chains may not run the whole length of the fibril, but that the latter is made up of an aggregation of smaller parts of quite definite size. In collagen the sub-unit has been considered to be a protofibril of size about 640×12 A., although recently Schmitt has proposed a unit of about 2000×50 A., which he has named 'tropocollagen'. Striations of axial lengths about 210 A. (particularly in developing material) and 70 A. are also observed in electron micrographs of collagen. It is of interest to note that evidence for structure of size approximately 200 A. is found in α -keratin and 230 A. in fibrin, although the recurrence of this figure may be no more than coincidental. We have obtained X-ray diffraction evidence from dry collagen fibres which also suggests that the predominant 640 A. period is divided into sub-units of length about 210 A. A. C. T. North, P. M. Cowan, J. T. Randall

From *Nature* 18 December 1954.

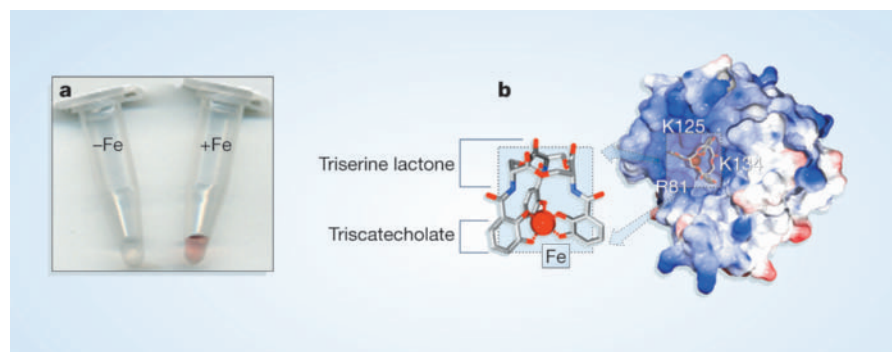


Figure 1 Snatching bacterial iron. **a**, In the presence of iron, solutions of the lipocalin 2 protein, synthesized in the laboratory, are distinctively *rosé*; without iron, they are colourless¹⁰. This demonstration led to the discovery that a ligand for lipocalin 2 is bacterial enterochelin — a secreted iron-chelating molecule. **b**, Bacterial enterochelin is composed of a triserine lactone molecule, which has three catecholate groups. These groups, between them, chelate an iron atom or ion. The iron-enterochelin complex can in turn be buried in the calyx of lipocalin, between three positively charged amino acids (R81, K125 and K134, where R is arginine and K is lysine). Flo *et al.*¹ have now found that lipocalin 2 is necessary for mice to keep bacterial infections in check; it works by stealing bacterial iron. (Molecular models courtesy of R. K. Strong, Fred Hutchinson Cancer Research Center, Seattle.)

Flo *et al.* reveal yet another mechanism: mammals can steal siderophores.

The authors first discovered that, during a bacterial infection, the mammalian liver, spleen and macrophages synthesize the lipocalin 2 protein, raising its concentration in the serum by log orders of magnitude. The lipocalins are a large family⁹ that have attracted structural biologists' attention because, although they vary in amino-acid sequence, their three-dimensional structures are remarkably conserved. They are made up of strands of so-called β -sheets, which form a barrel or a cup-like structure, or calyx, that carries small chemicals. A well-known example is the retinol-binding protein, with its retinoic acid ligand. So what is the chemical carried by lipocalin 2? Two years ago, Goetz *et al.*¹⁰ showed that iron-containing solutions of cloned lipocalin 2 are bright red (Fig. 1). Using several structural-analysis techniques, they tracked down the source of the red colour to the presence of iron-bound enterochelin.

The affinity of lipocalin 2 for enterochelin is very high (10^{-10} M), suggesting that this bacterial siderophore might be the authentic ligand (indeed, lipocalin 2 has been tentatively renamed siderocalin¹⁰). The idea is not so far-fetched — nitrophorin lipocalins (from the salivary gland of *Rhodnius prolixus*, the insect that spreads the parasite that causes Chagas' disease) were already known to carry iron-loaded haem groups¹¹. And lipocalin from human tears binds to bacterial and fungal siderophores¹².

Nonetheless, all of this might have been dismissed as an artefact of the cloning of lipocalin 2. Instead, it seems that it actually revealed an *in vivo* function. Using mice in which lipocalin 2 had been knocked out, Flo *et al.* unequivocally show that the protein is essential for limiting the growth of common bacteria that produce enterochelin, but does

not affect the growth of bacteria that rely on non-catecholate siderophores or other methods of iron acquisition. Eliminating lipocalin 2 increased bacterial colony counts by log orders of magnitude, and caused the rapid demise of the knockout mouse. Conversely, lipocalin 2 limited bacterial growth *in vitro* and *in vivo*, until reversed by excess siderophore. So lipocalin 2 directly regulates iron-dependent bacterial proliferation in mice — and probably, therefore, in humans.

These findings raise many issues for us to ponder. For instance, what happens to lipocalin 2 once it has chelated the siderophore and its iron? Recent work suggests¹³ that the kidneys filter and reabsorb the complex, recycling its iron. This pathway protects the kidney tubules from stress, hinting that the function of lipocalin 2 does not stop with stealing iron from microbes — the iron-loaded protein may also have other activities in mammalian cells. Indeed, lipocalin 2 is produced in massive amounts by cells that are damaged by chemicals or oxygen depletion in the absence of bacterial infection. This suggests that the production of lipocalin 2 is either a stress response that was originally intended as a defence against bacteria, or perhaps a mechanism for shuttling an unidentified endogenous ligand into cells. That endogenous ligand might even be a mammalian version of the bacterial siderophore, although the existence of such molecules has not been confirmed¹⁴.

Another possibility raised by the new findings is that many other lipocalins, whose functions are still unknown, are also iron carriers and provide an innate defence against microorganisms. And perhaps our most dangerous bacterial enemies, such as *Pseudomonas*, escape our control by making siderophores that are beyond the reach of our lipocalin-based surveillance mechanisms. Flo and colleagues' results¹ are an

open invitation to researchers to custom-design not new artificial iron chelators, but new siderophore chelators.

Great resourcefulness is required to capture insoluble iron. In the fight between mammals and microbes for this metal, it seems that, thanks to lipocalin 2, mammals are the winners — at least for now.

Jonathan Barasch is in the Departments of Medicine and of Cell Biology, and Kiyoshi Mori is in the Department of Medicine, Columbia University, New York City, New York 10032, USA.

e-mails: jmb4@columbia.edu

km2112@columbia.edu

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Archaeology

Much rowing for fish

Daniel Pauly

A thousand years ago, there was a shift in the fish diet in England from freshwater to marine species. The relevant case history, derived from picking through leftovers, has a contemporary resonance.

Aelfric (AD 955–c. 1020), the Abbot of Eynsham, near Oxford, has in his *Colloquy* a master asking a fisherman why he does not fish in the sea. The fisherman answers, “Sometimes I do, but rarely, because it is a lot of rowing for me to the sea”¹. Yet, in spite of all the rowing this transition required, Aelfric lived during the period when the fish that the English ate changed from mainly freshwater species to mainly marine ones — principally herring (*Clupea harengus*) and representatives of the cod family, the ‘gadids’, with cod (*Gadus morhua*) dominating. We know this from a study by Barrett and colleagues, just published in *Proceedings of the Royal Society*², which documents the contents of 127 ‘assemblages’ (of old fish bones) from settlements around England, covering a period from the seventh to the sixteenth century.

There is a large community of archaeologists and historians working up the material that was left over (in middens, old latrines and so on) from the meals taken by our ancestors. But this literature seems rarely to be used to draw inferences about the historical exploitation of natural resources, at least in the fisheries literature, which is a pity. Using such remains, Barrett *et al.* have identified the origin of intensive marine fishing, and hence a period that should serve as a baseline when evaluating the present state of marine ecosystems — at least in the waters around England.

The transition towards marine species, they suggest, was due to a decline in freshwater species. This decline was perhaps caused by pollution from mills and from agricultural run-off (farming was expanding during that period), and by overfishing of what are, after all, limited resources, relative to the demand

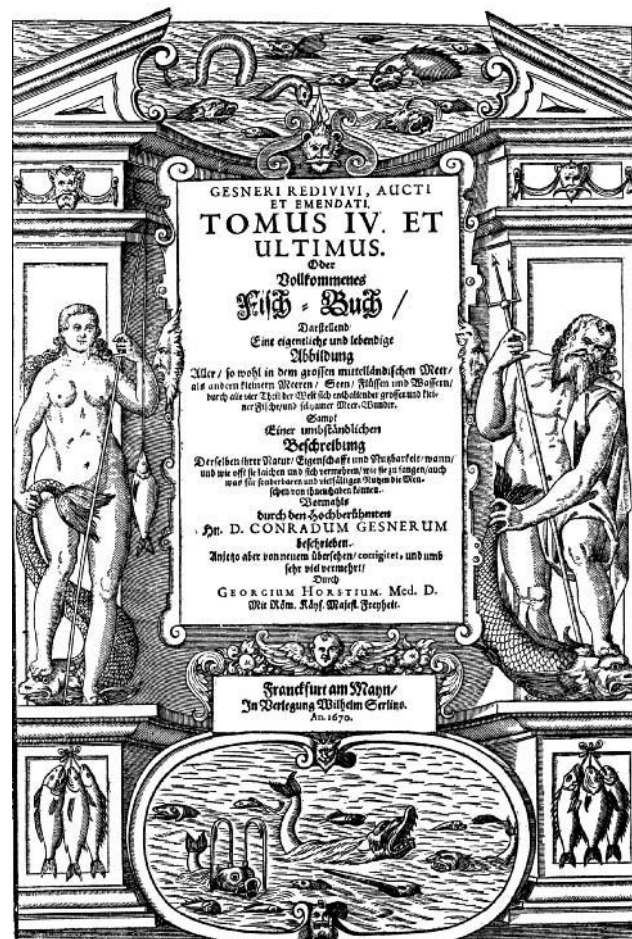
from a then-growing urban population.

Barrett *et al.* also point out that the period between 950 and 1050, which brackets the transition from freshwater to marine fishes in the English diet, was relatively warm. Such

conditions were not favourable to herring or cod in the seas around England, where both species are near the southern end of their ranges. Hence, only a massive (and historically undocumented) increase of trade with Norway, where the abundance of cod and herring does tend to increase with temperature, could link the observed dietary transition to shifting abundances resulting from environmental change.

In addition, Barrett *et al.* identify a group of ‘intermediates’ between strictly freshwater species and marine species. These are the flatfishes, which include species that migrate between marine and freshwater environments. And indeed, two flatfish species are mentioned by Aelfric’s fisherman, when he lists, upon being asked what he catches in the sea: “Herring and salmon, porpoise and sturgeon, oysters and crabs, mussels, winkles, cockles, plaice and flounders and lobsters and many similar things.”

There is another aspect of this study³ that the authors themselves don’t raise, but that I can’t resist mentioning here. This is that the analyses not only confirm and narrow down a freshwater-to-seawater transition previously known, though with less precise dates, from other parts of northwestern Europe (see ref. 1 for references), but they deepen our understanding of the last global transition towards the consumption of marine fish. This



Fish for all. Several centuries after the time of Aelfric’s fisherman, Conrad Gessner vividly described and depicted various species of European fishes in his *Fisch-Buch*, published in Frankfurt. It was part of the encyclopaedia that encompassed the zoological knowledge of his time.