OXOID™

Culture

Volume 19 No 2 September 1998 ISSN 0965-0989

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THE BARON JUSTUS VON LIEBIG MEMORIAL LECTURE 1997

Justus von Liebig – the gatekeeper of chemistry

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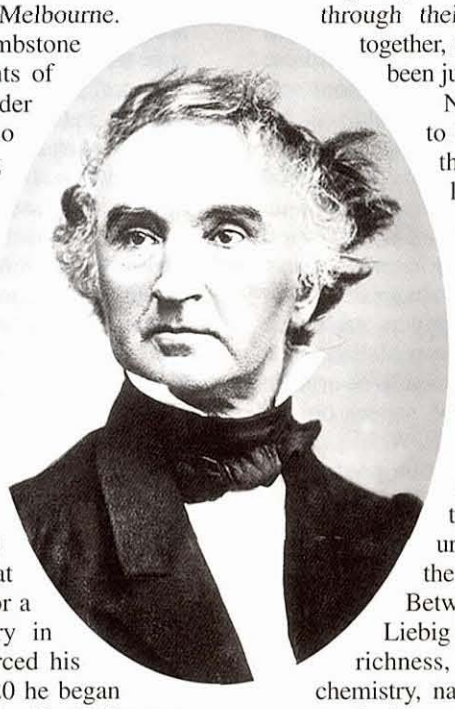
While travelling around Australia in 1989 I was repeatedly made aware of the presence of Justus von Liebig (1803-73). Near Alice Springs I came across Mt Liebig, in the Port Curtis district of New South Wales I learned of a Liebig County, as well as a Liebig Street in Warrnambool on the Great Ocean Road to the south west of Melbourne. And at Irrawang in New South Wales a tombstone commemorating the life and achievements of James King (1796–1857), a Scots freeholder who emigrated in the 1820s and who pioneered glass-making and wine growing in the state, mentioned his friendship with Liebig. Clearly, Liebig was a chemist with a world reputation, and as Mark Finlay has emphasised, he was one of the more significant nineteenth-century scientists in shaping an international vision of science and its enterprises.[1]



A Life in Chemistry

Liebig's father was a pigment and drug dealer in Darmstadt, where Liebig was born on 12 May 1803. After education at the Darmstadt Gymnasium, Liebig was for a short time apprenticed to an apothecary in Heppenheim, but financial difficulties forced his return to help in his father's shop. In 1820 he began the serious study of chemistry with Karl Kastner (1783–1857) at the Prussian University of Bonn, following Kastner to the University of Erlangen in Bavaria, where Liebig was awarded a doctorate *in absentia* in 1822. Through the patronage of Kastner, Liebig's diligence and brilliance had already been brought to the notice of the Grand Duke of Hesse Darmstadt and his ministers, who paid for Liebig to study chemistry in Paris between 1822 and 1824.

In Paris Liebig was greatly impressed by the lectures and researches of the French chemists Vauquelin (1763–1829), Thenard (1777–1857) and Gay-Lussac (1778–1850), with whom he came into daily contact. Liebig became determined to transplant their teaching methods and insights onto German soil. While in Paris Liebig investigated the dangerously explosive silver fulminate, a salt of fulminic acid. Cyanic acid was then being analysed in Berzelius's Stockholm laboratory by the young Friedrich Wöhler (1800–1882), who showed that its composition was identical with Liebig's fulminic acid. After the two young chemists had met at Frankfurt in 1826 and gone through their respective preparations and analyses together, each agreed that their original findings had been justified.

Not only did their little disagreement lead to the greatest friendship and partnership in the history of chemistry (over a thousand letters exchanged over their lifetimes have survived), but it was one of the principal factors which led Berzelius to announce the doctrine of isomerism in 1831—that two (or more) substances might have the same elementary composition, yet different properties, because their atoms were differently arranged.

By the time of Berzelius's announcement, of course, Wöhler had made an even more sensational discovery. In 1828, as he told Liebig excitedly, he had found that the urea extracted from a dog's urine had exactly the same composition as ammonium cyanate. Between them, and in the space of a decade, Liebig and Wöhler had revealed the source of the richness, fascination and difficulty of organic chemistry, namely that the simple elements of carbon, oxygen, hydrogen and nitrogen are capable of combining together in myriads of different ways to produce myriads of different compounds.

Ixodes ricinus – main vector of Lyme disease in western europe

Above: Liebig's analytical laboratory (after a drawing by Trantschold). Right: The same lab
Pictures courtesy of Wilhelm Lewicki, Liebigiana Ludwigshafen, Justus Liebig Society Gie

The work on fulminates, together with a meeting with the influential German traveller, Alexander von Humboldt (1769–1859), who then lived in Paris and who was always on the look out for young talent, led to Liebig's appointment to a Chair at the small university of Giessen in May 1824. As Liebig later observed in a fragmentary autobiography, *'at a larger university, or in a larger town, my energies would have been divided and dissipated, and it would have been much more difficult, perhaps impossible, to reach my goal.'*[2]

This goal was to expand and institutionalise the teaching of chemistry (which hitherto in German universities had been merely an adjunct of pharmacy for apothecaries and medical students) by placing it upon a thoroughly practical (or laboratory) basis and by concentrating attention upon the little-cultivated area of organic chemistry. As a teacher, Liebig had initially concentrated on pharmacy through a private school that the University authorities allowed him to conduct as part of his professorial duties. This school was eventually absorbed into the Philosophy Faculty in the 1830s. It was pharmacy and the difficulties with the analyses of the alkaloids, that directed Liebig's attention to the need to speed up and to simplify the basic copper oxide technique of organic analysis that he devised in 1830. The perfection of this method led to a decade of intensive investigation of organic compounds by Liebig and his students, Liebig himself publishing an average of thirty papers each year between 1830 and 1840. Several of these were to be of the greatest significance for the theory and practice of organic chemistry, notably an extensive series of papers on the nitrogen content of bases, the joint work with Wöhler on the benzoyl radical (1832), and on the degradation products of urea (1837), the discovery of chloral (trichloroethanol, 1832), the identification of the ethyl radical (1834), the preparation of acetaldehyde (ethanol, 1835), and the polybasic theory of organic acids (1838).

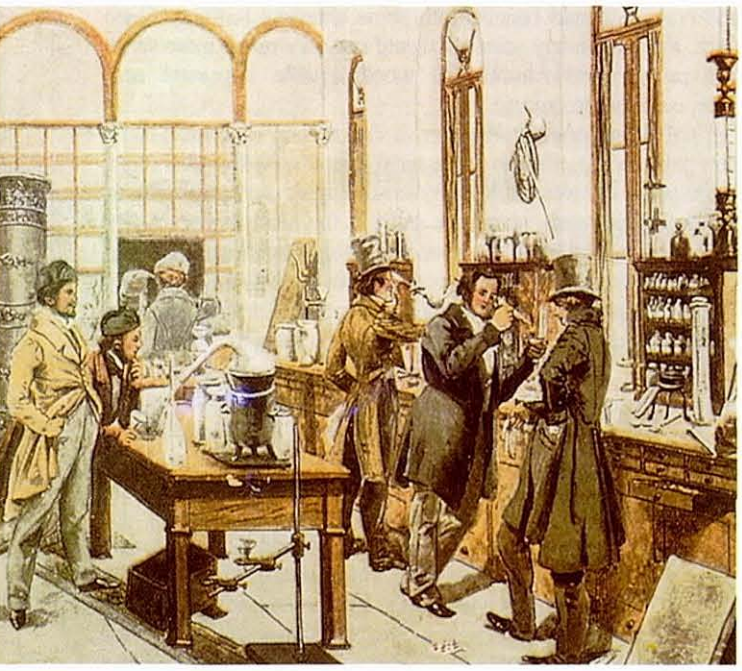
The radical theory, together with growing experience of organic analysis, gave Liebig and Wohler the confidence to analyse urine between 1837 and 1838 and to identify, analyse and classify its innumerable constituents and degradation products, such as urea (carbamide), uric acid, allantoin and uramil, which they believed to be produced by 'innumerable metamorphoses' of uric acid — itself a degradation product, as they supposed, of flesh and blood. This magnificent investigation, which galvanised British chemists when Liebig reported it to the British Association during his first visit to Britain in 1837, gave contemporary doctors a new insight into the pathology of kidney and bladder diseases. Later, in 1852, Liebig provided clinicians with simple chemical procedures for the quantitative determination of the urea in urine, and of the oxygen content of the air by its adsorption in an alkaline solution of pyrogallol (benzene-1,2,3-triol).

As more and more students flocked to Giessen in the 1830s — attracted by Liebig's techniques, reputation and the low fees made possible by the Hessen government's subsidy of the laboratory—Liebig had to expand his facilities and to systematise the training he gave to students. Two of his students, Heinrich Will (1812–90) and Carl Remigius Fresenius (1818–1897) were encouraged to publish textbooks on qualitative and quantitative analysis which were translated into several languages. Liebig's large number of foreign students (on average at least ten every semester) also ensured that his emphasis upon laboratory-based teaching and research was copied in other countries and German states—for example, at the Royal College of Chemistry founded in London in 1845, and Hermann Kolbe's large laboratory at Leipzig in Saxony in 1868.[3]

Liebig remained 28 years in Giessen, where the Duke of Hesse Darmstadt made him a Baron in 1845. In 1852, worn out by teaching, he moved to the University of Munich where he no longer gave practical instruction, but pursued his own interests and did a great amount of popular lecturing and writing.

Farming

Sickened by tedious disputes concerning the constitution of organic compounds, and convinced that organic chemistry could be used as a tool to investigate living processes, Liebig abandoned pure chemistry from about 1840 in an attempt to solve agricultural and physiological questions. In that year he published *Die organische Chemie in ihrer Anwendung auf Agricultur und Physiologie* (Braunschweig, 1840). Simultaneously translated into English and French, this book stressed that because 'perfect agriculture is the true foundation of all trade and industry ... a rational system of agriculture cannot be formed without the application of scientific principles.' Only the chemist, he claimed somewhat dogmatically, could tell the farmer the best means of feeding vegetables, the nature of the different soils and the action of different manures upon them. By analysing soils Liebig showed that the prevailing 'humus theory' in which a plant's carbon was supposed to originate principally from leaf mould, and not from the atmosphere by photosynthesis, was fallacious. On the other hand, Liebig argued incorrectly for many years that nitrogen in the form of atmospheric ammonia and nitrates in the soil were more important direct sources of plant nitrogen than manures, whose principal function he saw as providing trace minerals after



tory today. Left: Liebig's old laboratory of 1825.
and the Liebig Memorial Exhibition 1998.

decomposition in the soil. It was to provide these minerals more efficiently that in 1845 Liebig developed 'chemical manures'—the first of several commercial ventures made by him.

Agricutural chemistry led Liebig into a long and ill-mannered polemic with the wealthy landowner, Sir John Bennet Lawes (1814-1899), and his assistant, Joseph Gilbert (1817-1901), a former student of Liebig's at Giessen. Long-term field experiments on Lawes' estate at Rothamsted in Hertfordshire showed categorically that Liebig's fertilisers led to no marked improvement in crop yields. For some years Liebig refused to accept this conclusion and it was only after 1850, when John Thomas Way demonstrated the action of topsoil in withdrawing soluble salts from solution (ground absorption) that Liebig recognised that he had been mistaken to make his mineral manures difficult to dissolve.

Meanwhile, Lawes' independent researches led him to develop ammoniated and superphosphate fertilisers, a suggestion already made by Liebig in his Agriculturchemie. Their manufacture, which demanded a significant scaling up of sulphuric acid production, soon became an important accelerant of the industrialisation of Europe and of the vertical integration of chemical industries. Liebig's aphorism of 1843, that the measure of a country's civilisation lay in the amount of sulphuric acid it produced every year, has become famous. Both directly and indirectly, Liebig was an influential figure in the development of scientific agriculture and hence in increasing food supplies at a time when a rising European population was undergoing urbanisation and industrialisation.

Disease and Food

In 1842 Liebig published a sequel, *Die organischer Chemie in ihrer Anwendung auf Physiologie und Pathologie*. In this, by means of analyses and highly speculative equations, he attempted to unravel the metabolic routes by which foodstuffs were transformed into flesh and blood, and whereby tissues were degraded into animal heat, muscular work, and normal or abnormal secretions and excretions. His claim that fermentation and putrefaction were merely dynamic reshufflings of the constituent parts of chemical substances also led many doctors to espouse a chemical theory of disease which both challenged and made more sophisticated the predominant sanitarian view that disease was spread by a poisonous miasma which arose from accumulated sewage. Liebig's views of metabolism, pathology and disease also offered powerful propaganda for reformers who wanted to introduce practical chemistry and biochemistry into the medical curriculum. This is to be seen clearly in the pages of the British medical weekly, *The Lancet,* where Liebig's name is repeatedly found in the 1840s in admiring contexts.

Food

Liebig was much interested in the chemistry of food, especially in the best way to cook meat to preserve its nutritional qualities. In 1847, in a further book, *Chemische Untersuchungen über das Fleisch,* he described an 'extract of meat' (*extractum carnis*) prepared by low pressure evaporation of the soup from lean meat, and claimed that it would be a valuable restorative for the sick, wounded and ill-nourished. In later editions of his popular *Chemische Briefe,* or *Familiar Letters on Chemistry* as they were called in English, he pointed out that in countries where cattle were chiefly slaughtered for their hides or tallow (as in South America and Australia), such a food could be prepared extremely economically.

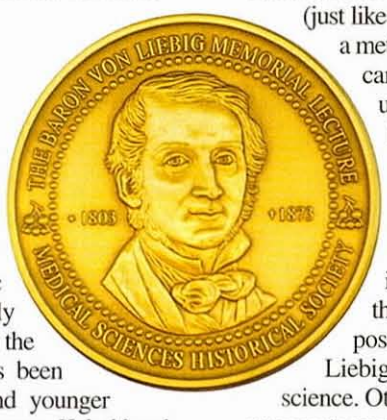
This suggestion was taken up in the 1860s by the German railway engineer, Georg Giebert, who with Liebig's promotional assistance marketed 'Liebig's Extract of Meat' as a nutritious food for invalids and the labouring classes. In the same decade Liebig also developed commercial processes for an artificial milk for babies, the improved baking of wholemeal bread, and for the silvering of mirrors.

Liebig and the British

Liebig enjoyed a particularly close relationship with Great Britain, which he visited six times and whose pattern of scientific education, agriculture and medical practice he helped to change. In 1843, several of his English friends tried to persuade him to apply for the vacant chair of chemistry at King's College, London, but the College's affiliation with the Church of England precluded any serious offer being made to him, and the position went instead to Liebig's erstwhile English pupil, William Allen Miller. Two years' later, when the Royal College of Chemistry was about to be established, it was again hoped that Liebig might personally come to London to superintend it and to recreate his Giessen teaching and research laboratory in Oxford Street; in the event, the post of Director was given, on Liebig's personal recommendation, to his best-known pupil, Hofmann (1818–1892), who spent twenty fruitful years in London. Together with Liebig's fifty or so other British pupils who held industrial or academic posts throughout the British Isles, Hofmann ensured that Liebig's name and work were kept prominently before the public eye. Liebig's name was also kept before the public through his commercial activities with the Liebig Extract of Meat Company and

Liebig's Concentrated Milk Baby Food. Liebig looked to Britain for a respectful and sympathetic audience for his ideas on, and discoveries in, chemical science. Here was an overseas audience which, though frequently provoked into controversy by his work, was often more sympathetic towards it than ones in the German nations. Equally, young professionalising groups of British chemists, doctors, engineers and educators, found 'Liebig' an effective publicity slogan, agent and figurehead in their campaigns to reverse the individualistic and utilitarian tendencies in British culture and society.[4]

Gatekeeper of Chemistry



Liebig could be irascible, pig-headed, quarrelsome, and sometimes devious, but he never became quirky, obstreperous and an embarrassment. Quarrels were quickly patched up, and by deliberately side-lining himself from the dreadful theoretical problems of organic chemistry after 1840, he avoided the frequently painful controversies over atomic weights and the issue of chemical structure, leaving what has been called 'the quiet revolution' to his pupils and younger colleagues such as Hofmann, Frankland, Williamson, Kekulé and Kolbe. Instead Liebig moved chemistry into the market place, into a socio-political context, by arguing and demonstrating its significance for the benefit of society in food production, nutrition and public health.

At Munich in 1852, he was able to relax from the punishing schedule of work he had kept up since joining Kastner as a student at Bonn and Erlangen. At Munich, through popular lectures and expansions of his readable *Chemische Briefe*, he was able to play the role of an elder statesman of science and to comment on broader issues such as scientific methodology, to oppose rank materialism, or, like a Hebrew prophet, warn the nations of the earth of the dangers of failing to recycle sewage or replace soil nutrients that were harvested as animal and human food. Above all, after signal failures in his prime to make money from chemistry, he was able to demonstrate, through the exploitation of the cadavers of South American cattle, how chemistry, or rather chemical physiology, could be exploited commercially. His success here was surely a model for the industrialisation of the food processing industry upon which twentieth-century society is rooted.

In my biography, Liebig is portrayed as a gatekeeper who acted as an entrepreneur and propagandist for the extension of chemistry's boundaries. For Liebig, chemistry was a mature science loosely separated or bounded by other disciplines. His essential message was that these adjacent fields of endeavour populated by workers trained in very different traditions of theory and practice would benefit from some chemical fertiliser. Once done, these boundary sciences would not only make more sense and prove more fruitful, but would enable important new interdisciplines to emerge.

Liebig's central message was so extremely successful that it now takes some effort to understand that despite the glamorous propaganda of twentieth-century cosmologists and physicists or molecular biologists, chemistry really is the most fundamentally central and useful of all the sciences. Liebig's aphorism that soap (just like sulphuric acid) is a measure of civilisation is really a metaphor of the fact that our modern complex societies cannot function without chemists and the understanding of chemistry.

Liebig's career, therefore, deserves attention today not only because he helped to transform the teaching of chemistry and created the modern research school, but because he transformed the ways doctors, pharmacists, physiologists and industrialists saw chemistry, and how chemists themselves saw their roles intellectually and as possessing 'civic worth' in a modern society. For Liebig, chemistry was the fundamental, or central science. Other sciences were diminished and distorted if their exponents were ignorant of it; while at the borderlands between chemistry and the other sciences there were new disciplines such as chemical physics, pharmaceutical chemistry, agricultural chemistry, physiological chemistry (or biochemistry) and industrial chemistry calling out for research and for able recruits. It was at these borderlands that chemists could make their greatest contributions to society.

Liebig died on 18 April 1873, and was buried in Munich's Sudfriedshof Cemetery. As gatekeeper of chemistry Liebig said of himself, as Lord Macaulay had once said of Francis Bacon, that: ***'He was not the maker of that road; he was not the discoverer of that road; he was not the person who first surveyed and mapped that road. But he was the person who first called public attention to an inexhaustible mine of wealth, which had been utterly neglected, and which was accessible by that road alone. By so doing he caused that road, which had been previously trodden only by peasants and higglers, to be frequented by a higher order of travellers.'***

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European tick-borne infections

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A large number of infections are known to be tick-transmitted zoonoses, i.e. infections transmitted from animals to humans. The present article focuses on those infections which are prevalent in large parts of Europe, i.e. Lyme borreliosis (LB), tick-borne encephalitis (TBE), ehrlichiosis and babesiosis, with special emphasis on the two latter, emerging infections.

LYME BORRELIOSIS

Lyme borreliosis (LB) is the most common arthropod-transmitted zoonosis in both Europe and North America. LB is caused by a new species of the genus *Borrelia* (family *Spirochaetaceae*), transmitted by hard ticks (*Ixodidae*) of the genus *Ixodes*. In Western Europe, the vector is *Ixodes ricinus* **(Figure 1).** Animal reservoirs are rodents but large animals, e.g. deer, cattle and horses, are important for the life cycle of the tick. LB has a wider clinical spectrum in Europe than in the US, due to the presence in Europe of at least three genospecies of *B. burgdorferi* sensu lato, i.e. *B. afzelii, B. garinii* and *B. burgdorferi* sensu stricto compared with the one genospecies, *B. burgdorferi* sensu stricto, in the US.

The most common clinical manifestations of Lyme borreliosis in Europe are erythema migrans (EM) and neuroborreliosis[1]. LB has been reported from all European countries but incidence data are mainly estimates, ranging from 3 per 100,000 in the UK to 120–130 per 100,000 in Austria and Slovenia. The infection shows a gradient of increasing incidence from west to east, although pockets of high-endemic areas are present also in low-endemic countries.

Disease incidence rates are also increased in certain occupations, e.g. forestry workers and in some recreational occupations, e.g. orienteers. Incidence rates are higher in males, presumed to be due to higher occupational risks and more outdoor activities. Children show both higher incidence rates and a different distribution of manifestations, e.g. more neurological disease, than adults.

Treatment strategies for LB vary between countries and physicians but several regimens are useful. Prevention is currently based on protection against tick-bite but two vaccines, both based on the outer surface protein A (Osp A), have been tested in the US and preliminary reports indicate ≥85% efficacy. It is likely that a vaccine will eventually be available also in Europe although production will be more complex since the vaccine will have to include Osp A from all three genospecies in Europe compared with the one in the US trial.

The most commonly used diagnostic method for LB is serology by ELISA, including antibody detection in cerebrospinal fluid, supplemented in some laboratories by immunoblot.

The European Union, recognising the need for a joint effort in the field of Lyme disease, supported a Concerted Action on Lyme Borreliosis (EUCALB). This network of scientists and clinicians from seventeen European countries investigated[2] many aspects of the disease and disease transmission then made the information on European LB available on the Internet (http://www.dis.strath.ac.uk/vie/LymeEU/).

TICK-BORNE ENCEPHALITIS (TBE)

The disease[3,4] is caused by tick-borne encephalitis virus (TBEV) of the family *Flaviviridae*, genus *Flavivirus* (formally group B arbovirus), a family including many other known arthropod-borne viruses, e.g. those causing yellow fever, dengue and Japanese encephalitis. Two subtypes of TBEV are recognised, one European and one Far Eastern, transmitted by *Ixodes ricinus* and *Ixodes persulcatus*, respectively. The distribution of the two related tick species overlap in north-eastern Europe but in general the vector of TBEV is *I. ricinus* in western and central Europe and *I. persulcatus* in Russia.

Tick-borne encephalitis is a major cause of morbidity in central, eastern and northern Europe. West of a sharp limit from mid Sweden though mid France, the disease is rare or non-existent. As for LB, disease incidence rates increase from west to east, with the highest reported rate of 184 per 100,000.

The majority of LB infections (60–70%) are subclinical. In clinical cases, the incubation period is 7–14 days and the first viraemic stage with flu-like symptoms lasts 2-8 days. This first phase is reported by 60–70% of patients who later go on to develop the second, neurological stage. The second stage is found in 5–30% of cases with clinical disease and the manifestations vary from pure meningitis to meningo-encephalitis, with or without paralysis. Reported mortality rates for the western TBEV infection vary between 0 and 4%.

Bob Gibbons, ARDEA London

Figure 1. *Ixodes ricinus* – main vector of Lyme disease (Lyme borreliosis) in western europe.

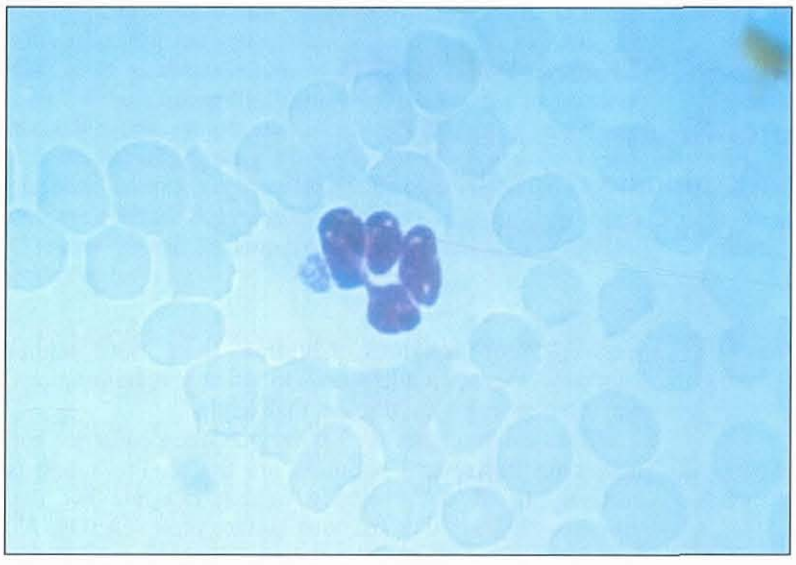

Figure 2. Characteristic inclusion body (morula) seen next to the nucleus of a granulocyte in a blood smear from a horse infected with the HGE agent. Morulae are diagnostic for granulocytic ehrlichiosis in humans also but infected granulocytes are much less of a common finding in human than in veterinary medicine.

The disease, in children, is relatively mild and both the severity of the disease and the risk of permanent sequelae increase with age. The reported rates of long-term sequelae vary between 2 and 11% for permanent paresis, 7–14% for hearing defects and 0–24% for cerebellar dysfunction. Carefully conducted follow-up studies indicate moderate to severe postencephalitic symptoms in 36–58% of patients.

Laboratory diagnosis of the disease relies on serology. The most widely used assay is a commercially available ELISA for IgM and IgG antibodies.

No specific treatment is available. Prophylaxis and post-exposure prophylaxis with specific immunoglobulin can be given up to the fourth day of presumed exposure. An efficient vaccine against TBEV is available from two manufacturers and is widely used in endemic areas. Vaccination should be offered to those who are habitually involved in outdoor work and leisure activities.

EHRLICHIOSIS

The genus *Ehrlichia* (family *Rickettsiaceae*), named in honour of Paul Ehrlich, consists of obligate intracellular pathogens that invade white blood cells (**Figure 2**). Ehrlichiae are known to cause disease in animals in Europe but were not known as human pathogens on this continent until recent research, conducted in the US, identified ehrlichiosis as an emergent, tick-borne zoonosis[5-7].

Monocytic ehrlichiosis

In 1986, infection with an *Ehrlichia* species was recognised in a patient at Fort Chaffee, Arkansas, US, by characteristic morulae (inclusion bodies) in circulating mononuclear cells and by a significant antibody response to *E. canis* antigen. The causative agent was isolated in 1991 and was shown to be closely related, but not identical to *E. canis*. The human monocytic ehrlichia was classified as a species of its own and named *E. chaffeensis*.

Infection in humans varies from subclinical to fulminant. In the US, many hundreds of clinical cases have been recognised. However, a majority of the infections seem, as for LB, to be subclinical, abortive or mild. The most common clinical manifestation is a flu-like illness with fever, malaise and myalgia. Common laboratory findings include leukopenia, thrombocytopenia, anaemia and elevated liver transaminases. The treatment of choice is doxycyclin.

Severe disease manifestations that have been reported are adult respiratory distress syndrome (ARDS), renal failure and severe hepatic disease. Serious neurological involvement is manifested by seizures, coma and CNS lesions. Persistent infection, a characteristic of ehrlichial infections in animals, has also been demonstrated. No cases of blood-transfusion transmitted infections are known.

Annual incidence rates of 3–5 per 100,000 have been estimated in the endemic areas of southern US. Infection rates in ticks of up to 32.5% have been reported.

In Europe, virtually nothing is known of either the incidence or the prevalence of the disease but a few serological cases of *E. chaffeensis* infection have been reported. No certain clinical case has been identified. The vector is unknown.

In the US, the vector most consistently associated with the disease is *Amblyomma americanum*, the lone star tick, which is not present in Europe. Ehrlichiae have been shown by PCR in adult *Amblyoma americanum* ticks (but not in nymphs) and in *Dermacentor variabilis* (the American dog tick) in one case.

The animal reservoirs and susceptible hosts are, as yet, not fully identified. The white-tailed deer is susceptible to experimental infection and populations of deer show high rates of antibodies to *E. chaffeensis* in endemic areas. Experimentally infected dogs, like the white-tailed deer, show persistent infection. The role of rodents in the transmission cycle remains to be elucidated.

Diagnosis is confirmed by PCR and serology by immunofluorescence (IF) with commercially available slides. Patients seroconvert during the second week of the disease and, unlike antibodies to LB, the antibodies measured by IF to *E. chaffeensis* return to base-line levels within 2 years. No ELISA has been described.

Granulocytic ehrlichiosis

In 1994, human infection with a granulocytic ehrlichia was described from the northern midwest US. IF and PCR demonstrated that the disease was caused by an agent related to or identical with *E. equi* and *E. phagocytophila*, two well-known agents of veterinary diseases both in the US (*E. equi*) and in Europe (both agents). The new agent shows a 99.8–99.9% homology to these agents, while the homology to the monocytic *E. chaffeensis* is only 92.5%. The agent of human granulocytic ehrlichiosis (HGE) is currently unnamed since it remains unclear whether it represents a new species or the human infection is caused by *E. equi/E. phagocytophila*.

Clinical disease is similar to that caused by *E. chaffeensis*, varying from subclinical to fulminant. The most common clinical manifestation of HGE is, as for *E. chaffeensis* infection, a flu-like disease. Granulocytic ehrlichial infection also causes thrombocytopaenia, leukopaenia, and elevated transaminases.

The estimated minimum incidence is 3 per 100,000. The vector is the same as for LB, *I. scapularis*, in eastern and mid-west US. The transmission cycle of the HGE agent has

not been fully elucidated. The white-tailed deer seems to represent a likely reservoir and the role of rodents remains to be clarified.

Human HGE infection in Europe was first identified by serology in LB patients from Switzerland, UK and Norway. A seroprevalence study[8] in a general population of south-western Sweden showed 21/185 (11.4%) HGE seropositives where the rate for LB seropositives was 25/185 (13.5%). Recently, four clinical cases have been found in Slovenia[9] but unpublished cases are known both from Scandinavia and other parts of Europe.

The presence of the HGE agent in Europe was first demonstrated in Swedish *Ixodes ricinus*[10]. The animal reservoirs have not yet been fully established but the HGE agent has been identified as a common cause of clinical disease in Swedish dogs and horses[11].

The diagnosis of HGE is based on PCR and serology by IF. Antibodies measured by IF seem to return, as for *E. chaffeensis*, to base-line level within two years of infection. Commercial slides using a recent human HGE isolate are available. No ELISA has been described.

BABESIOSIS

The causative agents, *Babesia* species (phylum *Apicomplexa*), named after Victor Babes, are pear-shaped, malaria-like, protozoan parasites. The piroplasms are represented by two families, the *Babesiidae* and the *Theileriidae*. The two differ in target cells and also in the mode of replication in ticks. Some of the agents currently classified as babesiae are more closely related to theileriae[12-14].

More than 100 species of *Babesia* are known but only two, *B. divergens* in Europe and *B. microti* in the US, have until recently been shown to cause disease in humans. Of these, *B. divergens* belongs to the classical *Babesia* while *B. microti* is a more *Theileria*-like organism. In 1991, a human pathogen, WA1, was found in California and shown to be a new species, related to but different from *B. gibsoni*. In 1996, a strain (MO1) resembling but not identical to *B. divergens* caused a fatal disease in a splenectomised patient in Missouri, US.

Babesia divergens

The first human case of babesiosis was described in 1957 from Yugoslavia, a fatal case of *B. divergens* infection in a splenectomised farmer. Since then, some 25 clinical cases of *B. divergens* infection, a majority in splenectomised patients, have been described from all parts of Europe. The mortality rate has been around 50%.

The disease is characterised by fever, myalgia, haemolytic anaemia and haemoglobulinuria, with renal failure and sometimes pulmonary oedema in the most severe cases. The disease has recently been successfully treated with quinine and clindamycin, in combination with exchange transfusion in severe cases.

Although *B. divergens* is an important pathogen in cattle all over Europe, *B. divergens* infection in immunocompetent individuals has not been investigated. A few serological studies, presented as case or congress reports, indicate the possibility of infection.

I. ricinus, the vector of *B. burgdorferi*, is also a vector for *B. divergens* but others cannot be ruled out. Cattle represent the obvious reservoir.

Diagnosis of *B. divergens* infection can be made on blood smears when many parasites are present (as shown in **Figure 3**). Inoculation of gerbils can also give the diagnosis. Serological diagnosis of *B. divergens* is made by IF on smears from experimentally infected animals. Antibodies seem to decrease to base-line levels within a year. *B. divergens* can be cultured in erythrocytes (**Figure 3**). Neither an ELISA for antibodies to *B. divergens* in human sera nor a PCR have been published.

Babesia microti

The European situation with a rare but severe infection in immunocompromised individuals differs markedly from the US, where many hundreds of cases of *B. microti* infection have been described, the majority in immunocompetent individuals.

Recent seroepidemiological studies in eastern US have indicated infection rates for *B. microti* varying from 2% in asymptomatic individuals to 7.5% in individuals with a history of tick bite and fever. Another population study showed seroprevalence rates for *B. microti* infection of 9% in adults and 12% in children.

In Europe, virtually nothing is known about *B. microti* infections. Sporadic reports have claimed asymptomatic seropositives for *B. microti* in Europe (France, southern Germany) but no systematic studies have been conducted. It is likely that the infection occurs in immunocompetent individuals also in Europe, especially as *B. microti* was isolated, several decades ago, from rodents in both Germany and the UK.

In the US, the most severe, often fatal, infections of *B. microti* have been described in splenectomised patients. *B. microti* causes more severe disease in older individuals than in children, a parallel to the clinical spectrum seen in veterinary medicine. The symptoms in immunocompetent individuals are uncharacteristic or flu-like as in ehrlichiosis, with malaise, fatigue, fever, headache, myalgia, arthralgia, vomiting, depression and emotional lability as major symptoms. Dark urine is common as are mild increases in transaminases. Babesiosis has been associated with a late-onset adult respiratory distress syndrome (ARDS). Chronic infections in untreated or inadequately treated patients are not uncommon.

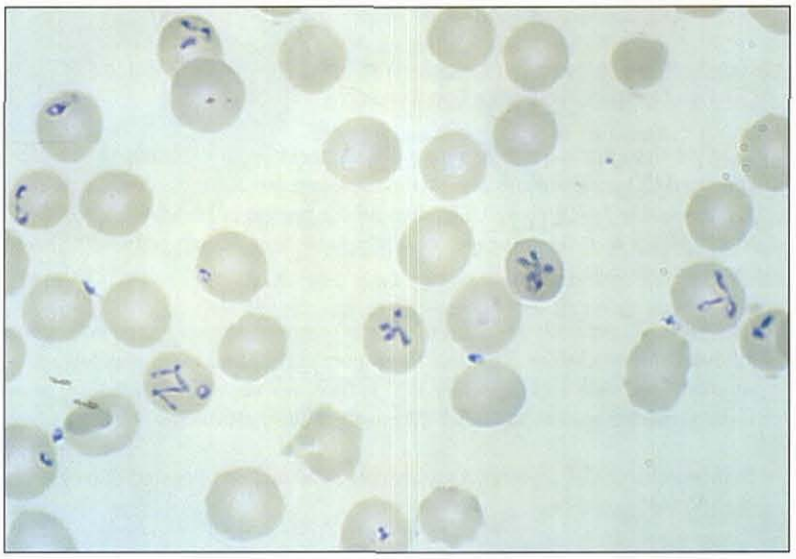

Figure 3. *Babesia divergens* infection in red blood cells. Note multiple forms (including the typical "Maltese cross") which will have to be differentiated from malarial parasites in blood smears. The high degree of parasitaemia, as shown in these organisms cultured in human red blood cells, is only found in immunocompromised patients.

Asymptomatic cases of *B. microti* appear to recover spontaneously but the severely ill patients need treatment with a combination of clindamycin and oral quinine, which seems to be the most effective regimen. Treatment of milder cases with doxycyclin is currently being investigated in the US.

Chronic infection in asymptomatic individuals with a low grade parasitaemia has been shown to be transmitted by blood transfusion. More than 20 cases have until now been documented from the US, including a fatal case of blood transmitted infection with a late-onset ARDS.

The vector for *B. microti* is *Ixodes scapularis* in the US, the same as for *B. burgdorferi* and for HGE. Rodents have been shown to be a major reservoir of *B. microti* in endemic areas. In Europe, one of the known vectors of *B. microti* is *I. ricinus*, alongside *I. trianguliceps*.

Slides for *B. microti* serology, unlike *B. divergens*, are commercially available. Antibodies decrease to base-line levels within a year in a majority of cases. PCR for *B. microti* is widely used in diagnosis of current infection. Attempts to culture *B. microti in vitro* have so far been unsuccessful. No ELISA for *B. microti* has been published.

Other babesioses

The clinical picture described for the WA1 has shown the same type and range of symptoms, from asymptomatic to severe clinical disease, as has been described for *B. microti*. The most severe clinical disease, as for other babesial infections, occurred in a splenectomised individual. The only case of MO1 was a fatal infection.

The presence of WA1 in Europe was suggested by a congress report but it has to be noted that the IF for WA1 gives a one-way, extensive cross-reactivity to other babesial species.

CO-TRANSMISSION AND CO-INFECTION

Cotransmission of two or several of these agents have been reported both from the US and from Europe e.g. for TBE and LB. As for the other agents, *Ehrlichia* and *Babesia*, clinical investigations in human medicine in Europe lag vastly behind the US. In veterinary medicine, however, the aggravating effect of concurrent infection of louping-ill virus (related to TBEV) and *E. phagocytophila* has been known for a long time. The aggravation is due to the immunosuppressive effect of both ehrlichial and babesial infections.

Co-infections with two or three tick-borne agents have recently been suggested in serological studies both from the US and from Europe. Seropositivity, especially in LB, does, however, not necessarily mean a current infection. Nevertheless, a well-designed study from the US showed that, in patients with clinical LB, concurrent *B. microti* seropositivity resulted in a more severe clinical picture with a longer disease duration than LB alone[15].

These tick-borne zoonoses and the role of co-infections need to be investigated in Europe and the network established within the Concerted Action on Lyme borreliosis intends to expand its scope of action to include all these tick-borne zoonoses in Europe. The newly founded European Society for Emerging Infections (ESEI), which will hold its 1st conference September 13-16, 1998, has also the goal of establishing interdisciplinary collaborations to promote international efforts for the study of these infections.

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