Request for an Opinion

Correspondence
Harold Neimark
neimah25@hscbklyn.edu

Phylogenetic analysis and description of *Eperythrozoon coccoides*, proposal to transfer to the genus *Mycoplasma* as *Mycoplasma coccoides* comb. nov. and Request for an Opinion

Harold Neimark,¹ Wallace Peters,² Brian L. Robinson²
and Lindsay B. Stewart²

¹Department of Microbiology and Immunology, Box 44, Morse Institute of Molecular Genetics, College of Medicine, State University of New York, 450 Clarkson Avenue, Brooklyn, NY 11203, USA

²Centre for Tropical Antiprotazoal Chemotherapy, Northwick Park Institute for Medical Research, Watford Road, Harrow, Middlesex HA1 3UJ, UK

Eperythrozoon coccoides, an eperythrocytic organism that causes a mild haemolytic anaemia in laboratory and wild mice, currently is thought to be a rickettsia. To determine the relationship of this agent to other haemotrophic bacterial parasites, the 16S rRNA gene of this organism has been sequenced and it is shown by phylogenetic analysis that this wall-less bacterium is not a rickettsia but actually is a mycoplasma. This mycoplasma shares properties with and is closely related to the other uncultivated mycoplasmas that comprise a recently identified group, the haemotrophic mycoplasmas (haemoplasmas). The haemoplasma group is composed of former *Eperythrozoon* and *Haemobartonella* species as well as newly identified haemotrophic mycoplasmas. Haemoplasmas parasitize the surface of erythrocytes of a wide variety of vertebrate animal hosts and are transmitted mainly by blood-feeding arthropod vectors. Because both primary infections and chronic latent infections caused by this bacterium have been observed in many laboratories and this bacterium has been the subject of much experimental work, considerable information exists about this haemotrophic mycoplasma that may be applicable to other haemoplasmas. It is proposed that *Eperythrozoon coccoides* be reclassified as *Mycoplasma coccoides* comb. nov. A Request for an Opinion is submitted to the Judicial Commission of the International Committee on Systematics of Prokaryotes regarding this reclassification.

Eperythrozoon coccoides is a blood parasite of laboratory and wild mice and was one of the first discovered members (Schilling, 1928; Dinger, 1928, 1929) of what has emerged as a large and ubiquitous group of haemotrophic bacteria that infect a broad range of mammalian hosts including, occasionally, humans (Weinman, 1935, 1944; Kreier *et al.*, 1992; Neimark *et al.*, 2001). *E. coccoides*, like most members of the group, is vector-transmitted and produces a primary acute infection which is followed by a persistent latent infection. Diagnosis has been made by detecting organisms on erythrocytes in Romanowsky-type or acridine orange-stained blood smears.

For decades the widespread occurrence of *E. coccoides* in laboratory mouse colonies, mouse tumour lines and

protozoa research stocks was an unrecognized and serious problem because inapparent infections invalidated the results of numerous studies (Eliot, 1936; Gledhill, 1962; Riley *et al.*, 1964; Baker *et al.*, 1971).

E. coccoides was thought to be a rickettsia because of its obligate parasitism, small size and filterability, erythrocyte localization and arthropod vector transmission. *E. coccoides*, together with a number of similar haemotrophic bacteria, was classified in the order *Rickettsiales*, family *Anaplasmataceae* (Kreier & Ristic, 1984; Kreier *et al.*, 1981, 1992). Recently, phylogenetic analysis of 16S rRNA gene sequences from several of these bacteria has demonstrated that these uncultivated wall-less bacteria are not rickettsia, but instead are mycoplasmas (Rikihisa *et al.*, 1997; Neimark & Kocan, 1997; Neimark *et al.*, 2001, 2002b). In addition, several new haemotrophic *Mycoplasma* species have been identified in wild and domesticated animals (Messick *et al.*, 2002; Neimark *et al.*, 2002b, 2004). Here we show that *E. coccoides*

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the Liverpool strain (Liverpool School of Tropical Medicine) of *Mycoplasma coccoides* is AY171918.

Blood from mice infected with *E. coccoides* was collected with heparin and diluted 1:3 in Alsever's/glycerol solution and cryopreserved at -70°C . The blood was thawed and 0.2 ml inoculated by the intraperitoneal route into *Eperythrozoon*-free, random-bred, albino Swiss mice that had been inoculated 2 days earlier with *Plasmodium yoelii* strain NS1100. By 2 days after the *E. coccoides* inoculation, there was a heavy *E. coccoides* infection and blood samples were collected in acid/citrate/dextrose and approximately 50 μl applied to FTA filter papers (treated filter paper for collecting and storage of blood or other biological samples for subsequent DNA analysis; Fitzco), air-dried and stored at ambient temperature until processed. Three-millimetre-diameter circles were punched from the filters and each circle was transferred to a PCR tube, washed at room temperature three times with 500 μl of FTA Purification Reagent (Fitzco) for 10 min each, and then washed twice

Mycoplasma 16S rRNA gene sequences were obtained from GenBank. Alignments were made with the program CLUSTAL W, adjusted manually, and phylogenetic analysis of the sequence alignment was carried out with the phylogenetic program package PHYLIP 3.51c as described previously (Neimark & Kocan, 1997; Neimark *et al.*, 2002a). The phylogenetic tree was constructed using the neighbour-joining method corrected for nucleotide substitutions by the Kimura two-parameter option with the transition/transversion ratio set at 2. The dataset was resampled 1000 times and the resulting bootstrap percentage values are indicated at the nodes of the tree shown in Fig. 1.

Phylogenetic analysis produced an evolutionary tree showing the relationship between *E. coccoides*, other haemotrophic mycoplasmas, representative species of the pneumoniae group and representatives of other mycoplasma groups (Fig. 1). The tree indicates that the *E. coccoides* 16S rRNA gene sequence clusters together with

all the other previously identified haemotrophic mycoplasmas on a branch related to the pneumoniae group of the genus *Mycoplasma* (Neimark & Kocan, 1997; Johansson *et al.*, 1999; Neimark *et al.*, 2001).

The high bootstrap percentage values at most of the branch points in the phylogenetic tree (Fig. 1) indicate the probability that this is a robust tree. However, the few low bootstrap values (values below 50) at some branch points suggest these branches may be due to chance. The phylogenetic tree shows that *E. coccoides* (*Mycoplasma coccoides* comb. nov.) falls in the haemofelis cluster of the haemoplasmas where it is related to, but is clearly distinct from, *Mycoplasma haemomuris* (85% similarity), a more pathogenic epierythrocytic mycoplasma found in laboratory rats and mice and in wild mice. Serum from mice infected with *E. coccoides* has been reported to react with *Haemobartonella muris* (*M. haemomuris*) and *Anaplasma marginale* antigens (Wigand, 1958). It is interesting to note that these two rodent mycoplasmas cluster phylogenetically with *Mycoplasma haemofelis*, a parasite of cats, which are rodent predators.

E. coccoides was first identified in laboratory mice (Schilling, 1928; Dinger, 1928) but wild mice probably are natural hosts (Bruymoghe & Vassiliadis, 1929a, b), and rats and rabbits have been infected experimentally (Bruymoghe & Vassiliadis, 1929c; Eliot & Ford, 1930). Natural infections with *E. coccoides* are transmitted by adults and nymphs of the lice *Polypax spinulosa* (Eliot, 1936) and *Polyplax serrata*, which can transmit an infection after fasting for as long as 24 h (Weinman, 1944; Berkenkamp & Wescott, 1988); there is no evidence of *E. coccoides* replication in lice and transmission appears to be mechanical. Fleas, the rat louse and the mites *Myobia musculi*, *Mycoptes musculus* or *Radfordia affinis* are not vectors (Eliot, 1936; Berkenkamp & Wescott, 1988). Experimental infections are produced usually by intravenous or intraperitoneal inoculation of infected blood and by oral administration of citrated blood but attempts at transmission by smearing mucous membranes (eyes, nostrils and urogenital surfaces) with citrated blood were not successful (Thurston, 1955).

Infections produce an intense parasitaemia that peaks on day 2 to 5 (Thurston, 1955; Gledhill *et al.*, 1965a; Peters, 1965; Ott *et al.*, 1967; Glasgow *et al.*, 1971, 1974) when the number of organisms can exceed 10^9 (ml blood)⁻¹ (Stansly & Neilson, 1965). Subsequently, the parasitaemia declines rapidly so by day 6 or 7 the number of organisms in the peripheral circulation is very low and organisms may go undetected in blood smears unless they are specifically searched for. The acute phase displays a haemolytic anaemia with the haematocrit falling from 50–55% to 20–30% but by day 8 haematocrit levels return to the normal range (Glasgow *et al.*, 1971). A marked splenomegaly is a characteristic of the infection and there is a general lymphadenopathy (Marmorston, 1935; Ansari *et al.*, 1963; Riley, 1964). There are no obvious clinical symptoms and mortality is rare.

A latent infection ensues which persists for months and possibly for the life of the animal but there is no recognizable disease. Splenomegaly also persists in carrier mice presumably as long as they are infected (Baker *et al.*, 1971) and low numbers of organisms may appear sporadically for brief periods in the peripheral blood of chronic carriers (Thurston, 1955). The existence of a latent infection is usually revealed by splenectomy which results in the reappearance of large numbers of organisms in the peripheral blood within 48 h; lower numbers of organisms then persist in the peripheral blood for several months (Schilling, 1928; Eliot & Ford, 1930; McCluskie & Niven, 1934; Marmorston, 1935; Thurston, 1954, 1955).

A notable finding is that *E. coccoides* infection raised phagocytic activity far above normal (Gledhill *et al.*, 1965a). Also, there is a marked suppression of the interferon response (by 80 to 95%) beginning 48 to 72 h after infection and continuing for 3 to 4 weeks (Glasgow *et al.*, 1971, 1974). The capacity to produce interferon returned to normal by 6 weeks (Glasgow *et al.*, 1971, 1974), so the interferon suppression lasted from about the end of the acute phase through the early latent phase. This response differs from a transient interferon response caused by *E. coccoides* infection (Glasgow *et al.*, 1971; Suntharasamai & Rytel, 1973) that was probably due to release of pre-formed interferon (Glasgow *et al.*, 1971).

Treatments that damage or stress the reticuloendothelial system (RES), such as X-irradiation, activated latent infection and in some instances infections were fatal (Furth, cited by Marmorston, 1935). Inoculating latently infected mice with *Borrelia recurrentis* or *Trypanosoma lewisi* (Dinger, 1929), malaria (Thurston, 1955; Ott *et al.*, 1967) or lymphocytic leukaemia cells (Marmorston, 1935) activated latent infection. Also, chemical agents, such as ethyl palmitate, which damage the spleen are known to activate latent infection in other haemoplasmas (Finch & Jonas, 1973).

During the acute phase infection there is a clear temporal relationship between the rapid disappearance of organisms from the blood and the rise of antibody (Hyde *et al.*, 1972; Glasgow *et al.*, 1974). In spite of this relationship, it is a question whether neutralizing antibody has a major part in maintaining latency since splenectomy results in rapid reappearance of organisms in the peripheral circulation. Although host defences contain the infection, there is a standoff between the host and the parasite: the host suppresses the parasitaemia and is resistant to superinfection (Thurston, 1955; Glasgow *et al.*, 1974), yet the host is not able to eliminate the organisms and the infection persists.

Undetected *E. coccoides* infections in laboratory mouse colonies, mouse tumour lines and related materials have resulted in numerous cases of anomalous experimental findings and led to recognition that *E. coccoides* can cause complex pathophysiological changes that affect the host response to infection and that concurrent infections with *E. coccoides* and another infectious agent can markedly

alter the course of infection. Examples include conversion of the benign hepatitis produced by mouse hepatitis virus MHV1 to a fatal infection by a concurrent infection with *E. coccoides* (Niven *et al.*, 1952; Gledhill, 1956, 1962; Seamer, 1967); the increase in mortality from lymphocytic choriomeningitis virus infection in mice concurrently infected with *E. coccoides* (Seamer *et al.*, 1961; Seamer & Gledhill, 1965; Niven, 1971); the 'spleen weight increase factor' and other effects encountered during tumour transmission studies in mice (Nelson, 1956; Stansly, 1965; Stansly & Neilson, 1965, 1966a, b); the discovery of the lactate dehydrogenase-elevating virus (Arison *et al.*, 1963; Riley, 1964, Riley *et al.*, 1964; Fitzmaurice *et al.*, 1972); and the discovery that *E. coccoides* infection increased the virulence of *Salmonella typhimurium* because normally harmless amounts of endotoxin produced high mortality in *E. coccoides*-infected mice (Gledhill & Niven, 1957; Niven, 1971).

In contrast, Peters (1965, 1987) showed that concurrent infections with *E. coccoides* and *Plasmodium berghei* resulted in a plasmodiostatic effect that permitted longer survival of mice. Analogous effects were found with *E. coccoides* and additional plasmodia (Ott & Stauber, 1967; Ott *et al.*, 1967; Voller & Bidwell, 1968; Cox, 1975; Suntharasamai & Marsden, 1969, 1970), with *Babesia rodhaini* (Peters, 1965) and *Trypanosoma brucei* (Bidwell & Voller, 1967). Also, *E. coccoides* protected mice from the lethal effects of Simliki Forest virus (Voller & Bidwell, 1968).

Finerty *et al.* (1973) showed that concurrent *E. coccoides* infection reduced the antibody response of mice to *P. berghei* and suggested antigenic competition could be responsible for this effect. Subsequently, Ljungström *et al.* (1974) showed that mice with an *E. coccoides* acute infection or inapparent chronic infection when immunized with sheep red blood cells (SRBCs) had enhanced IgM plaque-forming capacity (acutely infected mice also had enhanced IgG capacity); however, they noted the possibility could not be excluded that the increase in plaque capacity was due to cross-reactivity between SRBCs and an *E. coccoides* antigen.

The question has been raised as to whether some animal mycoplasmas, such as *Mycoplasma pneumoniae*, which can attach to erythrocytes *in vitro* and are known to enter the blood circulation can also attach to erythrocytes *in vivo* (Neimark *et al.*, 2001). Mucous membranes are the primary tissue habitat for animal mycoplasmas but whether haemotrophic mycoplasmas can also colonize mucous membranes is unknown.

E. coccoides has not been cultivated on artificial media. Derrick *et al.* (1954) found *E. coccoides* survived up to 9 days in embryonated chicken eggs but attempts at passage were unsuccessful; serial passages in embryonated chicken eggs have been reported (Seamer, 1959) but not confirmed. The standard procedure for determining whether a mouse colony was infected was to splenectomize a dozen 3- to 4-month-old mice and examine their blood daily (Gledhill

et al., 1965b). An indirect fluorescent-antibody test has been shown to be useful (Baker *et al.*, 1971) and scanning electron microscopy has been suggested to be useful (Augsten, 1982) but a PCR assay would be preferred, especially for studies of latency.

E. coccoides is readily killed by antiseptics and various chemical agents (Thurston, 1955; Stansly & Neilson, 1966b). The organism, like all mycoplasmas, is completely resistant to penicillin and other antimicrobial agents that target the cell wall. Tetracycline treatment is effective in reducing the number of organisms in the peripheral circulation but, based on experience with other haemoplasmas, this antibiotic probably does not eradicate the organism from infected animals. Organic arsenicals have been shown to be effective for treating *E. coccoides* (Bruymoghe & Vassiliadis, 1929b) and Neoarsphenamine (300 µg per 20 g mouse) eradicated active and latent infections in mice (Thurston, 1953); these drugs have proved generally useful for haemoplasma infections (Michel *et al.*, 2000).

It is proposed that *E. coccoides* be transferred to the genus *Mycoplasma* as *Mycoplasma coccoides* comb. nov., with the specific epithet '*coccoides*' retained for continuity. Because this proposed transfer raises the taxonomic problems described below, we submit a Request for an Opinion of the Judicial Commission of the International Committee on Systematics of Prokaryotes and ask (i) the current use of the genus name *Mycoplasma* be maintained and (ii) *Mycoplasma coccoides* be considered a legitimate name.

(i) *Eperythrozoon* (Schilling, 1928) predates *Mycoplasma* (Nowak, 1929), consequently under Rules 15, 17, 44 and 51b the name of the united genus should be *Eperythrozoon*. We request an exception to the rules that would require renaming *Mycoplasma* species to *Eperythrozoon*, since changing the names of more than 100 *Mycoplasma* species to *Eperythrozoon* (as well as changing the order and family names from *Mycoplasmatales* and *Mycoplasmataceae*) would produce enormous confusion. In support of this request we cite Appendix 8 which states 'In those cases where strict adherence to the rules of nomenclature would produce confusion or would not result in nomenclatural stability, exceptions to the rules may be requested of the Judicial Commission of the ICSB.' With respect to confusion, it should be noted that over the past few years the transfer of the five other *Eperythrozoon* and *Haemobartonella* species to the genus *Mycoplasma* and the naming of several newly discovered haemotrophic bacteria as *Mycoplasma* has been well accepted and appears to have caused no confusion among veterinarians, the community most affected.

In addition, transferring all *Mycoplasma* species to *Eperythrozoon* would produce perilous names (Rule 56a). Problems could arise with mycoplasmas that cause serious human disease and because some animal pathogenic mycoplasmas are subject to quarantine regulations, changing their names would require name changes in quarantine regulations and documents around the world.

When the genus name *Eperythrozoon* was created with *Eperythrozoon coccoides* in 1928, there were no molecular identification or electron microscopic methods so it is not surprising that investigators did not recognize the fundamental characters shared by this bacterium and the bovine pleuropneumonia organism, then already known for more than 30 years, and the mycoplasma causing contagious agalactiae identified 3 years earlier. The incorrect identification of this and other haemotrophic mycoplasmas as rickettsia (the genera *Eperythrozoon* and *Haemobartonella*) has long caused misunderstanding, so it is especially important that their genus name clearly convey their phylogenetic affiliation within the family *Mycoplasmataceae*.

(ii) Although *E. coccoides* clearly is a *Mycoplasma*, according to the Rules of the Bacteriological Code, the new combination *Mycoplasma coccoides* is illegitimate because *E. coccoides* is the type species of the genus *Eperythrozoon*. Ideally, this particular problem could be ameliorated by including in the proposed transfer the last remaining named species in the genus, *Eperythrozoon parvum* (all the other recognized species in the genus have been shown to be *Mycoplasma* species). However, this uncultured organism is not available and there is no assurance it can be found in a reasonable amount of time since one of us (H.N.) has been searching for a specimen for some years without success (requests to veterinary colleagues in North America and Europe to take a specimen if they encounter an infected pig have not resulted in any specimens). Except for three early papers on *E. parvum* 40 to 50 years ago, there has been only one other paper on this organism, nearly 25 years ago, and the description in Bergey's Manual is quite limited. Since all the other species of *Haemobartonella* and *Eperythrozoon* in Bergey's have now been shown to be *Mycoplasma* species, it seems likely *E. parvum* would prove to be a mycoplasma but there is no direct evidence and it cannot be determined when a specimen of this bacterium cannot be found and studied.

[After this manuscript was originally submitted we were advised to add a Request for an Opinion concerning these problems. As the revised manuscript was being submitted, Uilenberg *et al.* (2004) proposed on the basis of priority that all *Mycoplasma* species should be renamed *Eperythrozoon* but at the same time proposed waving the requirement that the order (*Mycoplasmatales*) and the family (*Mycoplasmataceae*) names be changed.]

Description of *Mycoplasma coccoides* comb. nov.

Mycoplasma coccoides (coc.coi'des. Gr. n. *coccus* a berry; N.L. n. *coccus* a coccus; Gr. n. *eidus* shape; N.L. adj. *coccoides* coccus-shaped).

Basonym: *Eperythrozoon coccoides* Schilling 1928 (Approved Lists 1980).

Organisms in peripheral blood are coccoid and about 350 nm in diameter. Their association with the erythrocyte

does not produce a noticeable indentation in the erythrocyte surface (Tanaka *et al.*, 1965). Many organisms also are free in the plasma, especially early in the infection. Ring-shaped cells commonly observed in stained smears are an artefact of the staining process which suggests these organisms are easily deformed from their coccoid form. The cells are filterable through at least a 360 nm mean pore size colloidal filter (Niven *et al.*, 1952). An eperythrocytic parasite of laboratory and wild mice, albino rats, hamsters and rabbits. Organic arsenicals (Neoarsphenamine; Oxophenarsine) are effective therapeutic agents that can eradicate a latent infection (Thurston, 1953; Thompson & Bayles, 1966; Powers *et al.*, 1969); tetracyclines only suppress infection. The organism can influence the course of viral or protozoan infection in experimentally co-infected mice. Reported from Europe, North and South America and Africa. The louse *Polypax spinulosa* is a vector. The organism has not been cultured on artificial media. Organisms in citrated blood remain infective for several hours at 16 °C and approximately 11 days at 3 °C (Thurston, 1955) and are maintained by animal passage, and storage at -70 °C. DNA G+C content (mol%) not determined. Genome size not determined.

Because this bacterium is uncultivated, no type strain is established. However, it is suggested that, where possible, chromosomal DNA from this and other uncultivated bacteria be deposited in a type culture collection to provide a type strain reference.

Acknowledgements

We thank Dr J. P. Euzéby and a journal referee for helpful taxonomic advice.

References

- Ansari, K. A., Neilson, C. F. & Stansly, P. G. (1963). Pathogenesis of infectious splenic enlargement in mice. *Exp Mol Pathol* **2**, 61–68.
- Arison, R. N., Cassaro, J. A. & Shonk, C. E. (1963). Factors which affect plasma lactic dehydrogenase in tumor bearing mice. *Proc Soc Exp Biol Med* **113**, 497–501.
- Augsten, K. (1982). Identification of *Eperythrozoon coccoides* by scanning electron microscope. *Z Versuchstierkd* **24**, 169–170.
- Baker, H. J., Cassell, G. H. & Lindsey, J. R. (1971). Research complications due to *Haemobartonella* and *Eperythrozoon* infections in experimental animals. *Am J Pathol* **64**, 625–632.
- Berkenkamp, S. D. & Wescott, R. B. (1988). Arthropod transmission of *Eperythrozoon coccoides* in mice. *Lab Anim Sci* **38**, 398–401.
- Bidwell, D. E. & Voller, A. (1967). The effect of *Eperythrozoon coccoides* on infections in mice. *Parasitology* **57**, 22P.
- Bruymoghe, R. & Vassiliadis, P. C. (1929a). L'*Eperythrozoon* *coccoides*. *Compt Rend Soc Biol Paris* **100**, 763–764 (in French).
- Bruymoghe, R. & Vassiliadis, P. C. (1929b). Contribution à l'Étude des *Eperythrozoon* *coccoides*. *Ann Parasitol Hum Comp* **7**, 353–364 (in French).
- Bruymoghe, R. & Vassiliadis, P. C. (1929c). Transmission des eperythrozoaires de la souris. *Compt Rend Soc Biol Paris* **102**, 939–940 (in French).

- Cox, F. E. (1975).** Factors affecting infections of mammals with intraerythrocytic protozoa. *Symp Soc Exp Biol* **29**, 429–451.
- Derrick, E. H., Pope, J. H., Chong, S. K., Carley, J. G. & Lee, P. E. (1954).** Observations on infection of mice with *Eperythrozoon coccoides* Schilling. *Aust J Exp Biol Med Sci* **23**, 577–582.
- Dinger, J. E. (1928).** Een nieuw microorganisme bij muizen. *Nederl Tijds v Geneesk* **82**, 5903–5905 (in Dutch).
- Dinger, J. E. (1929).** Näheres über das *Eperythrozoon coccoides*. *Zentralbl Bakteriol Abt I Orig* **113**, 503–510 (in German).
- Eliot, C. P. (1936).** The insect vector for the natural transmission *Eperythrozoon coccoides* in mice. *Science* **84**, 397.
- Eliot, C. P. & Ford, W. W. (1930).** *Eperythrozoon coccoides* in mice. *Am J Hyg* **12**, 677–680.
- Finch, S. C. & Jonas, A. M. (1973).** Ethyl palmitate-induced bartonellosis as an index of functional splenic ablation. *J Reticuloendothel Soc* **13**, 20–26.
- Finerty, J. F., Evans, C. B. & Hyde, C. L. (1973).** *Plasmodium berghei* and *Eperythrozoon coccoides*: antibody and immunoglobulin synthesis in germfree and conventional mice simultaneously infected. *Exp Parasitol* **34**, 76–84.
- Fitzmaurice, M. A., Riley, V. & Santisteban, G. A. (1972).** Biological synergism between the LDH-virus and *Eperythrozoon coccoides*: studies on the mechanism. *Pathol Biol (Paris)* **20**, 743–750.
- Glasgow, L. A., Odugbemi, T., Dwyer, P. & Ritterson, A. L. (1971).** *Eperythrozoon coccoides*. I. Effect on the interferon response in mice. *Infect Immun* **4**, 425–430.
- Glasgow, L. A., Murrer, A. T. & Lombardi, P. S. (1974).** *Eperythrozoon coccoides*. II. Effect on interferon production and role of humoral antibody in host resistance. *Infect Immun* **9**, 266–272.
- Gledhill, A. W. (1956).** Quantitative aspects of the enhancing action of *Eperythrozoon* on the pathogenicity of mouse hepatitis virus. *J Gen Microbiol* **15**, 292–304.
- Gledhill, A. W. & Niven, J. S. (1957).** The toxicity of some bacterial filtrates for mice pre-infected with *Eperythrozoon coccoides*. *Br J Exp Pathol* **38**, 284–290.
- Gledhill, A. W. (1962).** Viral diseases in laboratory animals. In *Problems of Laboratory Animal Disease*, pp. 99–113. Edited by R. J. C. Harris. New York: Academic Press.
- Gledhill, A. W. (1965).** Quantitative aspects of the enhancing action of eperythrozoon on the pathogenicity of mouse hepatitis virus. *J Gen Microbiol* **15**, 292–304.
- Gledhill, A. W., Bilbey, D. L. & Niven, J. S. (1965a).** Effect of certain murine pathogens on phagocytic activity. *Br J Exp Pathol* **46**, 433–442.
- Gledhill, A. W., Niven, J. S. & Seamer, J. (1965b).** Elimination of *Eperythrozoon coccoides* infection from mouse colonies. *J Hyg (Lond)* **63**, 73–78.
- Hyde, C. L., Finerty, J. F. & Evans, C. B. (1972).** Antibody and immunoglobulin synthesis in germfree and conventional mice infected with *Eperythrozoon coccoides*. *Am J Trop Med Hyg* **21**, 506–511.
- Johansson, K. E., Tully, J. G., Bolske, G. & Pettersson, B. (1999).** *Mycoplasma cavipharyngis* and *Mycoplasma fastidiosum*, the closest relatives to *Eperythrozoon* spp. and *Haemobartonella* spp. *FEMS Microbiol Lett* **174**, 321–326.
- Kreier, J. P. & Ristic, M. (1984).** Genus III. *Haemobartonella* Tyzzer and Weinman 1939, 143^{AL}; Genus IV. *Eperythrozoon* Schilling 1928, 1854^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 724–729. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Kreier, J. P., Dominquez, N., Krampitz, H. E., Gothe, R. & Ristic, M. (1981).** The haemotrophic bacteria: the families *Bartonellaceae* and *Anaplasmataceae*. In *The Prokaryotes*, vol. 2, pp. 2189–2209. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.
- Kreier, J. P., Gothe, R., Ihler, G. M., Krampitz, H. E., Mernaugh, G. & Palmer, G. H. (1992).** The haemotrophic bacteria: the families *Bartonellaceae* and *Anaplasmataceae*. In *The Prokaryotes*, vol. 4, pp. 3994–4022. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. Berlin: Springer.
- Ljungström, I., Hultdt, G. & Voller, A. (1974).** The effect of *Eperythrozoon coccoides* infection on the number of plaque forming cells in mice. *Acta Pathol Microbiol Scand [B] Microbiol Immunol* **82**, 911–913.
- Marmorston, J. (1935).** Effect of splenectomy on a latent infection, *Eperythrozoon coccoides* in white mice. *J Infect Dis* **56**, 142–152.
- McCluskie, J. A. W. & Niven, J. S. F. (1934).** The blood changes in rats and mice after splenectomy with observations in *Bartonella muris* and *Eperythrozoon coccoides*. *J Pathol Bacteriol* **39**, 185–196.
- Messick, J. B., Walker, P. G., Raphael, W., Berent, L. & Shi, X. (2002).** ‘*Candidatus Mycoplasma haemodidelphidis*’ sp. nov., ‘*Candidatus Mycoplasma haemolamae*’ sp. nov. and *Mycoplasma haemocanis* comb. nov., haemotrophic parasites from a naturally infected opossum (*Didelphis virginiana*), alpaca (*Lama pacos*) and dog (*Canis familiaris*): phylogenetic and secondary structural relatedness of their 16S rRNA genes to other mycoplasmas. *Int J Syst Evol Microbiol* **52**, 693–698.
- Michel, J. C., de Thoisy, B. & Contamin, H. (2000).** Chemotherapy of haemobartonellosis in squirrel monkeys (*Saimiri sciureus*). *J Med Primatol* **29**, 85–87.
- Neimark, H. & Kocan, K. M. (1997).** The cell wall-less rickettsia *Eperythrozoon wenyonii* is a mycoplasma. *FEMS Microbiol Lett* **156**, 287–291.
- Neimark, H., Johansson, K.-E., Rikihisa, Y. & Tully, J. G. (2001).** Proposal to transfer some members of the genera *Haemobartonella* and *Eperythrozoon* to the genus *Mycoplasma* with descriptions of ‘*Candidatus Mycoplasma haemofelis*’, ‘*Candidatus Mycoplasma haemomuris*’, ‘*Candidatus Mycoplasma haemosuis*’ and ‘*Candidatus Mycoplasma wenyonii*’. *Int J Syst Evol Microbiol* **51**, 891–899.
- Neimark, H., Barnaud, A., Gounon, P., Michel, J. C. & Contamin, H. (2002a).** The putative haemobartonella that influences *Plasmodium falciparum* parasitaemia in squirrel monkeys is a haemotrophic mycoplasma. *Microbes Infect* **4**, 693–698.
- Neimark, H., Johansson, K.-E., Rikihisa, Y. & Tully, J. G. (2002b).** Revision of haemotrophic *Mycoplasma* species names. *Int J Syst Evol Microbiol* **52**, 683.
- Neimark, H., Hoff, B. & Ganter, M. (2004).** *Mycoplasma ovis* comb. nov. (formerly *Eperythrozoon ovis*), an eperythrocyclic agent of haemolytic anaemia in sheep and goats. *Int J Syst Evol Microbiol* **54**, 365–371.
- Nelson, J. B. (1956).** An ascites tumor appearing during the passage of *Eperythrozoon coccoides* in mice. *J Exp Med* **103**, 743–752.
- Niven, J. S. F. (1971).** Alan Watson Gledhill. 4 June 1910–20 July 1967. *J Med Microbiol* **4**, 389–404.
- Niven, J. S. F., Gledhill, A. W., Dick, G. W. A. & Andrews, C. H. (1952).** Further light on mouse hepatitis. *Lancet* **2**, 1061.
- Nowak, J. (1929).** Morphologie, nature et cycle évolutif du microbe de la péripleurémie des bovidés. *Ann Inst Pasteur (Paris)* **43**, 1330–1352 (in French).
- Ott, K. J. & Stauber, L. A. (1967).** *Eperythrozoon coccoides*: influence on course of infection of *Plasmodium chabaudi* in mouse. *Science* **155**, 1546–1548.
- Ott, K. J., Astin, J. K. & Stauber, L. A. (1967).** *Eperythrozoon coccoides* and rodent malaria: *Plasmodium chabaudi* and *Plasmodium berghei*. *Exp Parasitol* **21**, 68–77.

- Peters, W. (1965).** Competitive relationship between *Eperythrozoon coccoides* and *Plasmodium berghei* in the mouse. *Exp Parasitol* **16**, 158–166.
- Peters, W. (1987).** *Chemotherapy and Drug Resistance in Malaria*, 2nd edn, vol. 1. London: Academic Press.
- Powers, K. G., Jacobs, R. L., Good, W. C. & Koontz, L. C. (1969).** *Plasmodium vinckei*: production of chloroquine-resistant strain. *Exp Parasitol* **26**, 193–202.
- Rikihisa, Y., Kawahara, M., Wen, B., Kociba, G., Fuerst, P., Kawamori, F., Suto, C., Shibata, S. & Futohashi, M. (1997).** Western immunoblot analysis of *Haemobartonella muris* and comparison of 16S rRNA gene sequences of *H. muris*, *H. felis*, and *Eperythrozoon suis*. *J Clin Microbiol* **35**, 823–829.
- Riley, V. (1964).** Synergism between a lactate dehydrogenase-elevating virus and *Eperythrozoon coccoides*. *Science* **146**, 921–923.
- Riley, V., Loveless, J. D. & Fitzmaurice, M. A. (1964).** Comparison of a lactate dehydrogenase elevating viruslike agent and *Eperythrozoon coccoides*. *Proc Soc Exp Biol Med* **116**, 486–490.
- Schilling, V. (1928).** *Eperythrozoon coccoides*, eine neue durch Splenektomie aktivierbare Dauerinfektion der weissen Maus. *Klin Wschr* **7**, 1853–1855 (in German).
- Seamer, J. (1959).** The propagation and preservation of *Eperythrozoon coccoides*. *J Gen Microbiol* **21**, 344–351.
- Seamer, J. (1967).** Some virus infections of mice. In *Pathology of Laboratory Rats and Mice*, pp. 537–567. Edited by E. Cotchin & F. J. C. Roe. Philadelphia: F. A. Davis.
- Seamer, J., Gledhill, A. W., Barlow, J. L. & Hotchin, J. (1961).** Effect of *Eperythrozoon coccoides* upon lymphocytic choriomeningitis in mice. *J Immunol* **86**, 512–515.
- Stansly, P. G. (1965).** Non-oncogenic infectious agents associated with experimental tumors. *Prog Exp Tumor Res* **7**, 224–258.
- Stansly, P. G. & Neilson, C. F. (1965).** Relationship between spleen weight increase factor (SWIF) of mice and *Eperythrozoon coccoides*. *Proc Soc Exp Biol Med* **119**, 1059–1063.
- Stansly, P. G. & Neilson, C. F. (1966a).** Distinctive behaviour of *Eperythrozoon coccoides* in plasma and spleen of infected mice. *Nature* **211**, 1203–1204.
- Stansly, P. G. & Neilson, C. F. (1966b).** Sedimentation of *Eperythrozoon coccoides*. *Proc Soc Exp Biol Med* **121**, 363–365.
- Suntharasamai, P. & Marsden, P. D. (1969).** Studies of splenomegaly in rodent malaria. I. The course of splenomegaly in mice infected with *Eperythrozoon coccoides*, *Plasmodium berghei* yoelii and the two infections combined. *Trans R Soc Trop Med Hyg* **63**, 64–70.
- Suntharasamai, P. & Marsden, P. D. (1970).** The effect of *Eperythrozoon coccoides* infection or splenectomy, or both, on the infection with a Peru strain of *Trypanosoma cruzi* in mice. *Trans R Soc Trop Med Hyg* **64**, 167.
- Suntharasamai, P. & Rytel, M. W. (1973).** Interferon response and interferon action in *Eperythrozoon coccoides* infection of mice. *Proc Soc Exp Biol Med* **142**, 811–816.
- Tanaka, H., Hall, W. T., Sheffield, J. B. & Moore, D. H. (1965).** Fine structure of *Haemobartonella muris* as compared with *Eperythrozoon coccoides* and *Mycoplasma pulmonis*. *J Bacteriol* **90**, 1735–1749.
- Thompson, P. E. & Bayles, A. (1966).** Eradication of *Eperythrozoon coccoides* with oxophenarsine in normal and drug-resistant lines of *Plasmodium berghei* in mice. *J Parasitol* **52**, 674–678.
- Thurston, J. P. (1953).** The chemotherapy of *Eperythrozoon coccoides* (Schilling, 1928). *Parasitology* **43**, 170–174.
- Thurston, J. P. (1954).** Anaemia in mice caused by *Eperythrozoon coccoides* (Schilling, 1928). *Parasitology* **44**, 81–98.
- Thurston, J. P. (1955).** Observations on the course of *Eperythrozoon coccoides* infections in mice, and the sensitivity of the parasite to external agents. *Parasitology* **45**, 141–151.
- Uilenberg, G., Thiaucourt, F. & Jongejans, F. (2004).** On molecular taxonomy: what is in a name? *Exp Appl Acarol* **32**, 301–312.
- Voller, A. & Bidwell, D. E. (1968).** The effect of *Eperythrozoon coccoides* infection in mice on super-imposed *Plasmodium chabaudi* and Semliki Forest Virus infections. *Ann Trop Med Parasitol* **62**, 342–348.
- Weinman, D. (1935).** *Les Parasites Érythrocytaires Révélés par la Splénectomie: Bartonella et Eperythrozoon*. Paris: Amédée Legrand (in French).
- Weinman, D. (1944).** Infectious anemias due to *Bartonella* and related red cell parasites. *Trans Am Philos Soc* **33**, 288–338, 350.
- Wigand, R. (1958).** *Morphologische, Biologische und Serologische Eigenschaften der Bartonellen*. Stuttgart: Georg Thieme (in German).