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Malaria - recent developments

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Introduction

The World Health Organisation estimates that worldwide there are 300–500 million clinical cases of malaria every year. Transmitted by mosquito (see life-cycle, **Figure 1**), this protozoan parasite is responsible for 1.5–2.7 million deaths annually, one million of which occur in the under-fives. Although endemic in some 90 countries in the tropics and sub-tropics, more than 90% of these infections occur in sub-Saharan Africa, an area where there is often poor access to health care services.[1]

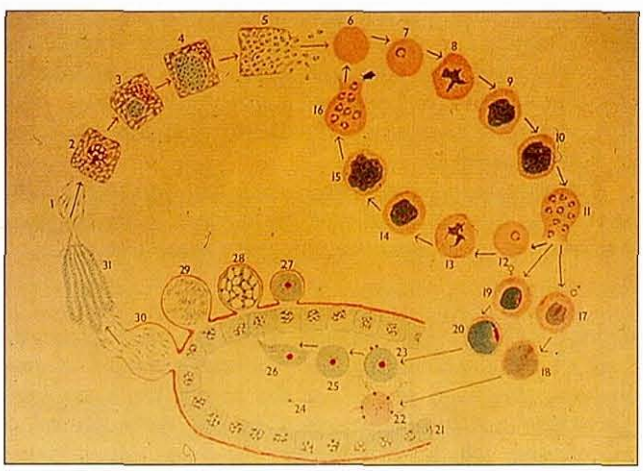

Figure 1. Life-cycle of mammalian malaria.
1 – Infective bite, 2-5 – Liver cycle, 6-11 and 12-16 – Intra-erythrocytic asexual cycle, 17-20 – Intra-erythrocytic sexual stages, 21-31 – Sexual cycle in mosquito gut.

Of the four species of malaria that infect man, only *Plasmodium falciparum* (**Figure 2**) is regularly fatal. The exact mechanism by which it does this is largely unknown but has traditionally been thought to be due to obstruction of the capillaries in the brain and other organs by parasitised red blood cells, a process known as sequestration (**Figure 3**). However, observations of complete recovery following long-term unconsciousness have led to an alternative hypothesis that cytokines, specifically tumour necrosis factor (TNF-α) and interleukin-1 play an important role in this process, particularly in the brain. It is suggested that they may have a role in inducing release of nitric oxide which acts on nearby synapses causing reversible coma.[2] The other species of malaria are responsible for varying degrees of morbidity, with *P. vivax* and *P. ovale* causing 'relapsing malaria' and *P. malariae* being responsible for mild clinical attacks up to 50 years after initial infection.

In the 1950s and 1960s, control of the disease focused on interruption of contact between human host and vector. Insecticide eradication programmes effectively interrupted transmission but insecticide resistance and local logistical problems gradually reduced the effectiveness of the control programmes and the incidence of malaria increased.[3] Malaria is, nevertheless, a curable disease provided that prompt diagnosis and treatment are available.

Diagnostic Techniques

Examination of stained blood smears has long been the gold standard for diagnosis of malaria infection. A properly stained and expertly examined film enables both species diagnosis and estimation of parasitaemia. However, despite this simple technology, indirect costs are expensive. Microscopical diagnosis requires not only a skilled operator but adequate infrastructure to maintain quality assurance as well as to ensure maintenance of supplies and equipment.[4]

Also in this issue (page 5):

Dr Theodor Escherich (1857–1911): Bacteriologist and Paediatrician

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An historical paper on this outstanding paediatrician and bacteriologist

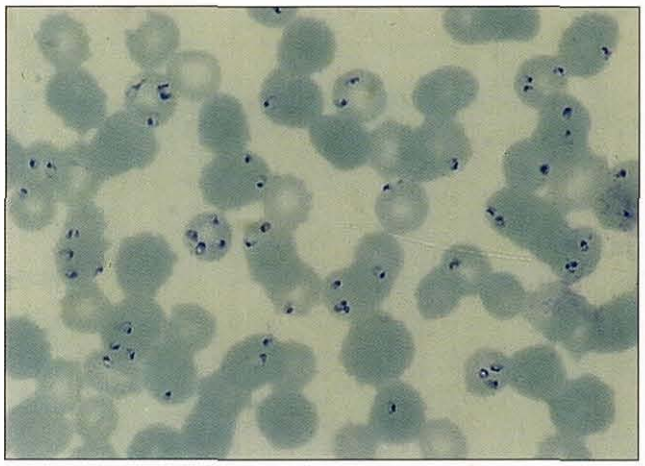
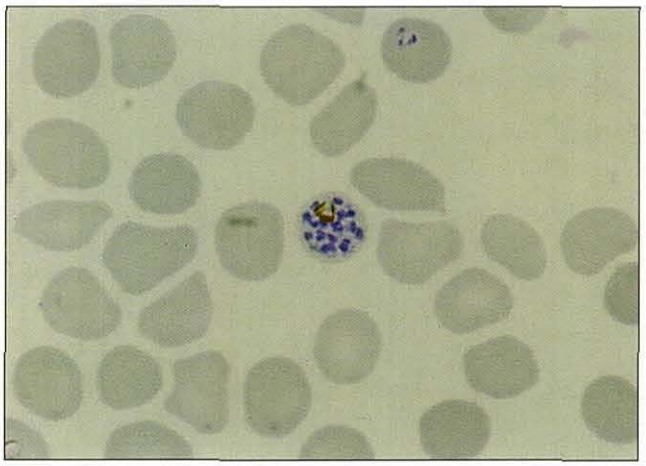
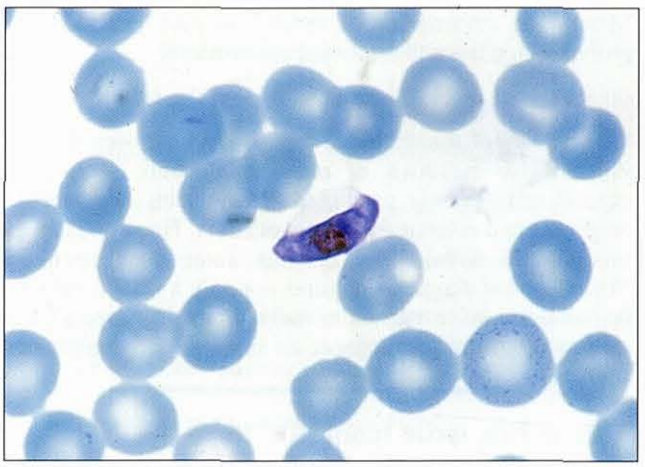


Figure 2
(a) Heavy infection of *P. falciparum* trophozoites



(b) *P. falciparum* schizont



(c) *P. falciparum* gametocyte

Consequently a simple and cost effective method which achieves the levels of sensitivity and specificity of blood film diagnosis is desirable.

The first diagnostic method to be commercially available was the Quantitative Buffy Coat (QBC), a method that involves the differential centrifugation of the blood sample. Visualization of the concentrated parasites is by acridine orange staining of their nuclei and examination *in situ* under a fluorescent microscope. The sensitivity is reported to be as good as a thick blood film for *P. falciparum* but appreciably less for the other species.[5] It is still necessary to use a blood film for species diagnosis and also for parasitaemia estimation. Later stages of the parasite are also difficult to identify as they tend to localise within the white blood cell layer. Although it is easier to train workers to use QBC the logistical requirements of microscopy still apply.

Alternative methods focus on the identification of parasite-specific molecules such as antigens and nucleic acids. Recently a specific assay for *P. falciparum* based on the detection of a histidine-rich protein (HRPII) has become commercially available.[4] This antigen is found both in the membrane of infected red blood cells and, as it is actively secreted from these cells, within the plasma of infected individuals. The levels of HRPII increase during the life cycle with a maximum reached during schizont rupture. The test depends on the capture of the HRPII by high affinity monoclonal antibody attached to a nitrocellulose and fibre dipstick. Bound HRPII is visualised by dye-labelled anti-HRPII polyclonal antibody.[5] In a recent multicentre study in the UK the ParaSight F test (Becton Dickinson) had a sensitivity of 95% and specificity of 93%.[6] However, detectable levels of HRPII may persist for several days after successful elimination of viable parasites. There have also been some false negative results even when parasite numbers have exceeded 1000/µl[4]. These antigen detection systems require minimum training and are proving of value in 'on-call' situations and for detection of low levels of *P. falciparum* in mixed infections. A similar assay for *P. vivax* is under development.[4]

There are numerous nucleic acid detection methods for the diagnosis of malaria, the majority of which are more applicable to large scale epidemiological surveys than to clinical diagnosis. Most involve radio-labelling and few are species specific. The polymerase chain reaction (PCR) has improved the sensitivity of these methods and new colorimetric labelling techniques have made them more applicable to field use. One group has developed a test that is both sensitive and specific for all four species by targeting the DNA sequence that encodes 18SrRNA.[7] A biotinylated PCR product is hybridized with a digoxygenin-labelled probe of which there is one for each malaria species. The hybrid product is then captured on a streptavidin-coated microtitre plate and visualised using a peroxidase-labelled anti-digoxygenin antibody and appropriate substrate. This test detected a parasitaemia of 0.0001% which is equivalent to 4 parasites per µl.

Unfortunately, however good the sensitivity and specificity of new diagnostic tools, cost is the ultimate limitation in those areas where malaria is most prevalent.

Chemotherapy

Since the 17th Century, quinine, which is extracted from the bark of the cinchona tree, has been the mainstay of anti-malarial drugs. In the 1930s the search for a synthetic alternative to quinine resulted in the development of chloroquine, which was effective, cheap and safe, followed by proguanil and pyrimethamine. Unfortunately, by 1960, foci of chloroquine resistant *P. falciparum* were developing in Venezuela and Thailand. Today, of those countries where *P. falciparum* is endemic only those of Central America have no recorded resistance to chloroquine.[1] In fact there are reports of resistance to nearly all the currently used anti-malarials, including quinine.

Mefloquine was originally developed in the 1970s for treatment of malaria with the hope that its reserved use would prevent build-up of resistance. Unfortunately because of the increase in chloroquine resistance it was soon used prophylactically and today is the prophylactic drug of choice for areas where there is increased transmission of chloroquine-resistant falciparum malaria. However its choice as a prophylactic has recently been the focus of controversy due to the neuropsychiatric nature of some of its side effects. Resistance to mefloquine has developed in

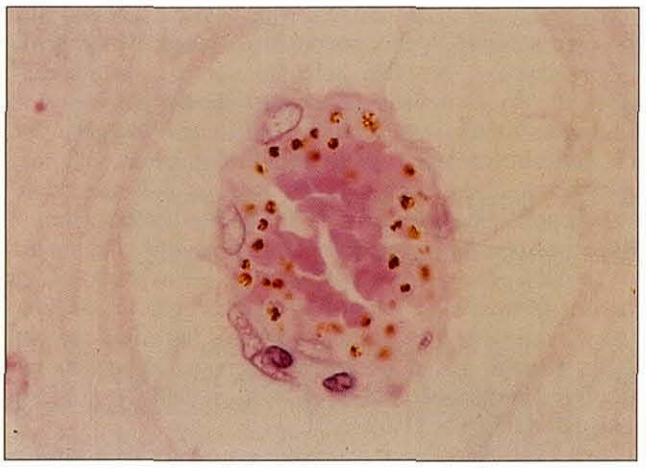


Figure 3. Cross section of a capillary in the brain showing sequestered parasitised red blood cells attached to the wall.

many countries, and in some areas of Thailand more than 50% of *P. falciparum* infections no longer respond to mefloquine.[1] Doxycycline is now advised for malaria prophylaxis in these multi-drug resistance areas.[8]

Decreases in sensitivity to quinine in Brazil and Southeast Asia has led to use of artemisinin and its derivatives for treatment of malaria. Artemisinin is derived from the plant *Artemisia annua L.* and has long been used in traditional Chinese medicine. Its more soluble derivatives, artemether and artesunate, provide satisfactory alternatives to quinine for the treatment of severe malaria[9,10] and have been successfully used in combination with mefloquine for the treatment of multi-drug resistant *P. falciparum*.[11,12] However there is some question concerning possible long term neurological side-effects of artemether.[13]

Currently there are a number of potential anti-malarial drugs in various stages of development. These include atovoquone, pyronaridine, alternative antifolate combinations, and also, analogues of the hypnozoitocidal drug, primaquine.[14] Atovoquone has broad spectrum anti-protozoal activity and *in vitro* studies have shown activity against both primary liver and erythrocytic stages of *P. falciparum*. Its mode of action as an electron transport inhibitor sets it apart from other anti-malarials and thus it is hoped that cross resistance will not occur. Initial studies have shown a recrudescence rate of approximately 30%[14] and so the use of atovaquone in combination with other anti-malarials has been investigated. When used in combination with proguanil, chosen because of its long safety record and the fact that it can be administered to children and during pregnancy, it successfully treated 101 out of 104 (97%) patients in Thailand with no adverse side effects.[15] Of all the new antimalarials this combination provides us with the most likely commercial candidate and is currently undergoing phase III trials.[14]

More novel approaches to anti-malarial therapy include the use of 'resistance-modifiers'[14] and anti-adhesion therapy.[16] 'Resistance modifiers' work on the basis that chloroquine-resistant parasites do not accumulate the same levels of the drug seen in chloroquine-sensitive parasites. These agents would reverse the rapid drug efflux seen in the resistant parasites and thus re-establish the effectiveness of the drug. As yet, none of the potential drug candidates have worked *in vivo*. Anti-adhesion therapy would work to minimize the complications of severe malaria. Peptides and/or antibodies specific for ligands or receptors would prevent sequestration by blocking binding sites. The use of the anti-TNF-α drug pentoxyfylline, which inhibits TNF-α release from macrophages, also seems promising.[13]

The problem of increasing drug resistance, in terms of both treatment and chemoprophylaxis and also the limited number of 'new' anti-malarials at an advanced stage of development, inevitably focuses attention on alternative methods of control. For more than 20 years hope has been pinned on the development of a malaria vaccine.

Vaccines

Malaria has a complex life-cycle and at any stage during its development is capable of expressing different surface antigens which may undergo clonal antigenic variation. This, coupled with a poor understanding of the exact mechanism behind naturally acquired immunity, has hampered the search for a vaccine. Ideally a vaccine would target different stages of the life-cycle and induce both T and B-cell responses. It would be both protective and boosted by natural exposure. Putative vaccines are categorised according to the developmental stage at which they are aimed; pre-erythrocytic, asexual or sexual (also known as transmission-blocking).

Pre-erythrocytic vaccines would theoretically avert infection, so effectiveness must therefore exceed 90% in order to prevent disease.[17] If effective, a pre-erythrocytic stage vaccine might be of use as a prophylactic for travellers from non-endemic areas.[3] Initial hopes centred on the attenuation of sporozoites by irradiating infected mosquitoes. Although protective immunity was induced it required a large number of infective bites,[18] was short-lived and species specific.[3] This type of immunity was reproduced by recombinant or synthetic expression of part of a naturally immunodominant antigen, the circumsporozoite protein (CSP); in particular a region of tandem repeats consisting of four amino acids, asparagine-alanine-asparagine-proline (NANP). Small trials using this tandem repeat were disappointing as it appeared that they were producing a way by which the parasite could evade the host immune response.[18] There is now also evidence to suggest that CSP is in fact released from the parasite before invasion of the hepatocyte.[17] An alternative candidate antigen is LSA-1 (liver stage antigen-1) which is synthesized during schizogony. Currently in experimental and early clinical studies are a number of other pre-erythrocytic antigens, individually or in combination, which are expressed in vectors including yeast, *Salmonella typhi*, vaccinia and influenza viruses.[18]

Malaria infection in infants in hyperendemic areas leads to high levels of morbidity and mortality but with increasing age and exposure both the severity and number of clinical episodes decreases. This immunity is unfortunately non-sterilizing and needs constant boosting by reinfection to maintain protective levels. An ideal asexual stage vaccine would mimic this, decreasing the level of parasitaemia and reducing disease severity. There are a number of asexual vaccine candidates including two merozoite surface antigens and the apical membrane antigen, all of which are undergoing safety and immunogenicity trials in humans and efficacy trials in monkeys.[3] Perhaps the best known and certainly the only one to reach full scale clinical trials is SPf66, the so-called 'Patarroyo vaccine'.

In 1987, following successful immunization of Aotus monkeys with three asexual stage purified proteins, Patarroyo used a combination of three synthetic peptides derived from the original proteins used to immunize monkeys and found complete or almost complete protection.[19] SPf66 is a polymer which consists of these three synthetic peptides intercalated with NANP repeats. Initial trials have seen thousands of South Americans vaccinated. Acute safety has been established with a reported efficacy of between 38.8 and 60.2% according to

age group, following natural exposure to malaria in these low transmission areas.[17]

Outside S. America, three large scale trials are reported to date. The first, in Tanzania where transmission is perennial and intense, saw 586 children aged 1 to 5 years immunized with three doses of either SPf66 or placebo. The vaccine was found to be both safe and immunogenic with an estimated efficacy of 31%.[20] A second trial in The Gambia[21] where transmission is moderate and seasonal, saw quite different results. 630 infants aged 6–11 months were vaccinated with no resulting protection against clinical attacks of malaria despite the presence of high titres of specific antibody. The most recent of the large scale trials has been in Thailand,[22] where 1221 children aged 2–15 years were vaccinated and followed for 15 months. These children have on average one clinical attack of falciparum malaria every 2–3 years. Therefore pre-existing immunity is low and symptomatic malaria is seen in all ages. This study showed no evidence that SPf66 provided significant protection against falciparum malaria in these children despite seroconversion of more than 70%. The reasons for these disparate results may be a reflection of age differences, levels of pre-existing immunity or genetic variations between the study populations and/or parasites. Whatever the reason, it is clear that SPf66 as it stands is not the answer and that improvements and/or alternative candidates should be sought.

Transmission-blocking vaccines would prevent mosquitoes from being infected thereby controlling disease in communities.There are two approaches, the first targets gamete antigens and because these antigens are expressed by circulating gametocytes, would be boosted by infection. The second targets ookinete antigens.[18] A yeast recombinant form of such an ookinete antigen (Pfs25) has been approved for Phase I clinical trials following successful experiments in rodents and monkeys. It induces transmission blocking antibodies that pass into the mosquito midgut as part of the bloodmeal and inactivate the developing ookinete.[3]

Alternative vaccine candidates include NYVAC Pf7 which is currently undergoing phase I and IIa trials. This vaccine is based on the expression of DNA coding for a combination of pre-erythrocytic, asexual and sexual antigens, in attenuated vaccinia.[18] Anti-disease vaccines would inhibit sequestration and decrease production of cytokines by targeting binding proteins and ligands, thus decreasing the incidence of the symptoms associated with severe malaria.[17] Possession of certain Human Leucocyte Antigens (HLA) has been shown to confer protection against severe malaria. Parasite antigens which when expressed with these particular HLA epitopes produce a protective cytotoxic T-cell response, are also potential vaccine candidates.[18] Most recently a study that investigated the high incidence of malaria in α-thalassemic children in Vanuatu[23] suggests that a significant increased susceptibility to infection with non-lethal *P. vivax* in these children may be inducing cross-species protection against severe falciparum malaria. Thus *P. vivax* may be acting as a natural vaccine in this community.

The lack of new anti-malarials to replace existing ones in the face of increasing resistance, together with the ongoing search for an effective vaccine brings us back full circle to vector control; use of insecticide-impregnated bednets has led to a significant reduction in child mortality in a recent national trial in The Gambia.[24]

Experience has shown that when confronted by a parasite with the capacity to evolve rapidly in an adverse environment, intermittent use of any diagnostic or intervention regime may result in a build-up of resistance and ultimately, increases in morbidity and mortality. Sustained financial support and stable infrastructure are therefore vital, a fact which should be remembered by those engaged in the fight against one of the world's most pressing infectious problems.

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Dr Theodor Escherich (1857–1911): Bacteriologist and Paediatrician

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During the First International Congress of Bacteriology in Paris (1930), *Bacterium coli commune* was renamed *Escherichia coli* for Dr Theodor Escherich (**Figure 1**). In the years since its discovery the organism has become important in medical bacteriology, bacterial genetics and as a tool in biotechnology. It is, therefore, remarkable that so little is known about its discoverer[1].

Escherich was an outstanding paediatrician and bacteriologist who combined science and medicine in a manner that has only relatively recently become commonplace. His contributions to medicine were not only founded on scientific principles, but proved seminal for the study of infectious diseases. The range of clinical problems dealt with in his publications is quite astonishing. Acuity of analytical observation, in the light of a training in laboratory science, gives the reader of his papers the feeling that he is in the presence of a 'modern' master.

Beginnings

Theodor Escherich was born on 29 November 1857 in Ansbach, a small town in the mid-Frankonian region of Bavaria. His father, Ferdinand Escherich, was a distinguished medical statistician and his mother, Marie Sophie Frieder, was the daughter of a Bavarian army officer, Baron Carl Stromer von Reichenbach. It seems that the young Theodor was a lively child with a tendency for troublesome pranks, and he was sent as a boarder to the famous Jesuit seminary and school 'Stella Matutina' in Feldkirch, Austria. The disciplines enforced there seem not to have had an adverse effect on the young schoolboy.

Escherich began his medical studies in Strasbourg in 1876 and, as was the practice in Germany at the time, he attended a number of medical schools, including the universities of Kiel, Berlin and Würzburg. He qualified at the University of Munich in 1881 with an MD thesis on *"Die marantische Sinusthrombose bei Cholera Infantum"* (Marantic sinus thrombosis in cholera infantum). The term 'cholera infantum' had been coined in 1733 by the American physician Benjamin Rush (1745-1813) for what is now known as infantile gastro-enteritis. It was finally shown in the late 1940s that certain strains of *E. coli*, now known as enteropathogenic, are responsible for the disease. It is a strange coincidence that Escherich's first publication was about a disease due to the organism he was later to describe. Though it is not entirely clear, it may well be that Escherich later suspected that some cases of diarrhoea in infants were due to his organism.

The making of a paediatrician

A year after he qualified, Escherich was appointed First Assistant to Carl Gerhardt, a general physician in Würzburg, with a specialist interest in paediatrics, who edited a notable nine-volume 'Handbook of the Diseases of Children'. Gerhardt aroused in Escherich a passionate interest in the diseases of children, but facilities for a training in paediatrics were not then available in Germany and Escherich went to Vienna where he spent some time with Hermann von Wiederhofer at the St Anna Children's Hospital. In 1885 he was appointed Clinical Assistant to Heinrich von Ranke, Professor of Child Health in Munich.

The making of a bacteriologist

A year before Escherich went to Vienna, Gerhardt had sent him to study the cholera epidemic then raging in Naples. In the same year he published a paper on cholera in which he confirmed Robert Koch's observations about the nature of the cholera bacillus but, following the views of his teacher, Pettenkoffer, Escherich had doubts about the cholera stool as a source of contagion. His Naples experience led Escherich to think that the bacteriology of the infant bowel might be of interest. This view was confirmed in Munich, where Wilhelm Frobenius, one of Koch's pupils, taught him Koch's principles and techniques of pure culture, and the methods for the characterisation and identification of bacteria[2].

The discovery of Escherichia coli

In the year of his appointment to the University of Munich (1885), Escherich read a paper to the Munich Society for Morphology and Physiology on "The Bowel Bacteria of the Newborn and Breast-fed Infants"[3]. In it he pointed out that at birth the meconium is sterile, but that bacterial colonisation of the infant bowel is very rapid and takes place within a few hours, including via the anus. In this paper Escherich described a number of organisms he had isolated from the infant stool. In particular, he characterised and described the two bacteria he had discovered, *Bacterium coli commune* (**Figure 2**) and *Bacterium lactis aerogenes*. They were later renamed *Escherichia coli* and *Klebsiella pneumoniae*. The descriptions

Figure 1. Photograph of Theodor Escherich by Ferdinand Meyer of Graz. Probably taken before 1902. (*Courtesy of Institut für Geschichte der Medizin, University of Vienna*).

included the cultural characteristics of these organisms in liquid and solid media, including not only classical solid media, such as gelatine and potato, but also agar, which had been introduced into bacteriology only three years earlier by Mrs Angelina Hesse[4]. Escherich provided a clear description of both organisms as facultative anaerobes and he showed that their growth under anaerobic conditions depends on the availability of fermentable carbohydrate. Escherich described *B. coli commune* as a harmless 'parasite', at a time when the term commensal had not yet been introduced.

At about the same time Escherich showed that the breast milk of healthy women is sterile, while that of lactating women with fever contains 'yellow' staphylococci. He also demonstrated that the staphylococci responsible for furunculosis of infants, invade by way of skin glands and on this basis he recommended its treatment with antiseptics.

The 'great' infectious diseases

In the late 19th century the infant mortality rate was 23 per cent, and diphtheria, scarlet fever and tuberculosis were major causes of childhood morbidity and mortality; Escherich made major contributions to the study of each of these infections.

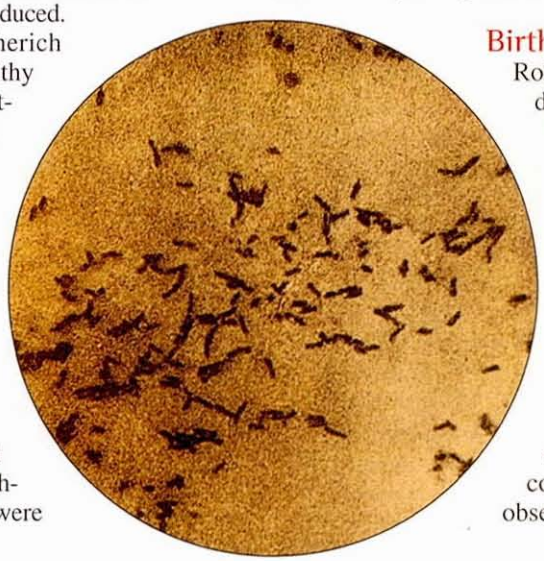

Figure 2. The first illustration of *Bacterium coli commune*. Coverslip preparation stained with gentian violet in aqueous aniline. (From Escherich T. (1886). Die Darmbakterien des Säuglings und ihre Beziehung zur Physiologie der Verdauung. In Arbeiten aus dem Pathologischen Institut zu München. pp. 1-180. Feredinand Enke: Stuttgart)

Diphtheria became one of Escherich's central interests from 1889. As a first step he confirmed Friedrich Loeffler's observation in 1884 that the disease is due to the diphtheria bacillus. At about this time Joseph O'Dwyer (1841-98) of Cleveland, Ohio, perfected a method of intubation designed to ease the breathing of patients with laryngeal diphtheria and in 1891 Escherich defined the criteria for the intubation of children. Two years later he distinguished diphtheria from other similar diseases on the basis of bacteriological and clinical criteria and in the same year he was the first to find diphtheria antitoxin in the blood of convalescent children. The suggestion had been made that diphtheria might be treated with antitoxin given by mouth or per rectum rather than parenterally, but Escherich showed that antitoxin given in this way was not absorbed. He finally summarised his work in a monograph "Diphtheria, Croup, Serum Therapy" (1895).

In 1890 Escherich was appointed extraordinary Professor of Child Health at the University of Graz, Austria, and director of the State Children's Clinic. This was under rather conservative management and modestly housed. Apart from his clinical and teaching duties, and to support his work, Escherich exercised his charm and determination to raise funds for the clinic. He found himself in a small provincial hospital and in time changed it into a noted institution in which his numerous pupils were active, including many from abroad. He said of himself that he had to "milk state, communal and private sources" for funds. With the funds he gathered he founded a laboratory and a special diphtheria department to study the management of the disease. Growth of public confidence in the work of Escherich at the Clinic, led to a growth in out-patient attendances from 3,000 to 10,000 and in-patient admissions from 15,000 to 26,000 per annum.

In Graz, Escherich married Margarethe, daughter of a distinguished physicist, Leopold Pfaundler; their marriage was a particularly happy one and they had one son and one daughter. The son died tragically of acute appendicitis while still a lad.

At about the time of his arrival in Graz, Escherich started to work on infantile tetany, a condition associated with muscle spasm and a low plasma calcium concentration. Escherich distinguished tetany clearly from similar but unrelated conditions and demonstrated its relationship to parathyroid deficiency.

Birth of the tuberculin reaction

Robert Koch claimed in 1890 that he had discovered the cure for tuberculosis, but he did not at the time disclose its nature. In the following year Koch let it be known that his discovery was 'old tuberculin'[5]. He supplied the tuberculin to only a selected group of physicians, one of whom was Escherich, who gave tuberculin by subcutaneous injection to some of his young tuberculous patients. He showed it was ineffective and that it actually made some children even more ill. In the course of the treatment trial Escherich observed reddening and induration at the site of the tuberculin injection. The phenomenon had been observed previously, but he gave it the name "Stichreaktion" (prick reaction); Escherich appears briefly to have taken an interest in the phenomenon and then seems to have forgotten it. Later when the specificity of the reaction became evident, interest was awakened and Clemens von Pirquet, a pupil of Escherich who succeeded him in the Vienna chair in 1911, defined the tuberculin reaction.

Bowel bacteriology again

Eventually in 1890-4 Escherich returned to his early interest in the bacteriology of the infant bowel. He suggested that *Pseudomonas (pyocyanea) aeruginosa* and enterococci can be the cause of bowel disturbances in babies. In 1894 he showed that *B. coli commune* is the cause of childhood urinary tract infections and suggested that in young girls it is an ascending infection. As part of his studies at about this

Figure 3. The St Anna Children's Hospital. An etching by Josephine Elbogen made before 1904. (Courtesy of Institut für Geschichte der Medizin, University of Vienna).

Figure 4. The St Anna Children's Hospital as it is today.

time Escherich cultured a bacillus from children suffering from dysentery. It was very similar to *B. coli commune*, but it did not produce gas when fermenting carbohydrates. It seems that Escherich thought the actual cause of dysentery, which he termed colicolitis, was *B. coli commune*. He therefore discarded the anaerogenic organism he had isolated and in doing so he left it to Kiyoshi Shiga (1870-1959) to discover the dysentery bacillus in 1898.

The Vienna chair

When Hermann von Widerhofer, the Professor of Child Health at the University of Vienna, died in 1902 the electors unanimously called Escherich to the chair and to be Director of the St. Anna Children's Hospital (**Figure 3**). This was the third independent children's hospital in Europe founded in 1837, only the Hôpital des Enfants Malades in Paris (1802) and the St. Nicholas Children's Hospital in St Petersburg (1834) were older foundations.

At the time of his appointment to Vienna, Escherich was promised a new children's hospital, but the necessary funds were not provided. So, in the first place, he set about adapting the old hospital by building laboratories and an infant department. After much effort over several years, involving lengthy discussions with government and the drawing up of plans, the funds necessary to build the new hospital became available. As fate would have it, Escherich was to see his dream only as an incomplete shell. The St Anna Children's Hospital, now run by the Red Cross, still continues in the service of children (**Figure 4**) and the new children's pavilion of Vienna General Hospital is named for Escherich.

The organiser

With Escherich's arrival in Graz new abilities and interests became apparent. He developed an interest in preventative paediatrics and showed extraordinary organisational and administrative abilities. While still a young doctor in Munich he had suggested that local city authorities should be responsible for rendering milk safe by sterilisation; 25 years later in Vienna he was to put his idea into practice. He was concerned to improve children's hospitals and encourage co-operation between fellow paediatricians and between these and other medical specialities. With seniority it seems that Escherich became more interested in grand plans than minor administrative achievements. He founded an organisation to distribute milk and provide advice for mothers; von Pirquet described it as a 'jewel'.

Escherich died on 15 February 1911. In a tribute one of his pupils said that the achievements of Theodor Escherich were so great that his colleagues could not imagine German paediatrics without him. A street in the 19th district of Vienna is named after him.

Escherichia coli continues to attract the attention of bacteriologists more than a century after its discovery[6]. The spectrum of disease for which it is responsible is remarkable — few organisms are more versatile. What Escherich would have made of the recent Scottish outbreak of *E. coli* 0157 infection must be left to our imagination but there can be no doubt that he would have been in the forefront of its investigation.

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