by **Professor B. Cooke** (Royal Free Hospital). He pointed out that there was considerable morphological and functional heterogeneity amongst Leydig cells, those adjacent to tubules about to enter stage VII spermatogenesis being the most active. This spermatogenetic stage is totally testosterone dependent, requiring local concentrations about 100-fold higher than those in the systemic circulation. As in the ovary and endometrium, some factors produced by stage VII germinal epithelium seem to tell the local population of Leydig cells to make large amounts of testosterone, though what these might be is not known. Growth factors or inhibin related peptides, again? At what level could such factors stimulate steroidogenesis? Options include the LH receptor itself (now cloned and sequenced), the transduction system, or the steroidogenic enzymes themselves (many now cloned too). So there are many possibilities and much work for the research biochemists, although this work still seems a long way from being translated into clinical benefit for the infertile male with oligoaesthenospermia. Even IVF results are very poor with his sperm (10–15% at best). Undoubtedly it is quality of sperms/semen that counts and not quantity since we were told by **Dr S. Shalet** (Manchester) of very good fertility results following the treatment of male hypogonadotrophic hypogonadism with hCG/HMG even with very low sperm counts ($< 2 \times 10^6$/ml).

Stress is known to be associated with reduced testicular function—indeed junior hospital doctors have been shown to have lower testosterone levels throughout their duty days compared with off-duty days. How may such a complicated situation be translated into altered Leydig cell function? The rate determining step in steroidogenesis, whether in testis, ovary or adrenal glands, is uptake of cholesterol into the mitochondrion, where reside the steroidogenic enzymes. This uptake process is mediated by a labile protein(s), and recently benzodiazepine receptors have been described on the outer mitochondrial membrane, although the endogenous ligand is as yet unknown. A further, older observation is the ability of the testis to synthesise pro-opiomelanocortin-derived peptides, especially beta-endorphin. Could there be opiate receptors too on testicular Leydig cell mitochondria and could beta-endorphin, either systemic or testicular in origin, be a ligand? And might not this provide a link between stress in doctors (and others) and reduced testosterone? This is highly speculative, but at least testable.

Cell–cell crosstalk

A recurring theme throughout the symposium was the issue of communication between cells of different functional and anatomic compartments within reproductive tissues. Endocrinologists and reproductive biologists have been at the forefront of the mushrooming fields of paracrinology and autocrinology. In a system so crucial for survival of the species it is self-evident that the orchestra must play in perfect harmony. We are now learning that not only the classical instruments but some novel ones as well are called upon to assist in the performance.

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Infectious diseases

A joint conference of the Royal College of Physicians and the Royal College of Pathologists. held on 13th and 14th December 1990.

First day

In the past ten years, the specialty of infectious diseases has had a new lease of life, which has been associated with a greater demand for the expertise of communicable disease physicians and microbiologists. This change is not simply due to the advent of newly identified diseases such as acquired immuno-deficiency syndrome (AIDS), legionnaire's disease, and toxic shock syndrome, but has also arisen as a result of new problems with old diseases such as tuberculosis and malaria, the expansion of the immunosuppressed patient population, and the growing awareness of a need for rational antibiotic prescribing. The conference was a timely appraisal of new developments in this rapidly changing specialty, and paid particular attention to advances in our understanding of the molecular biology and pathophysiology of infection. The conference affirmed a multidisciplinary approach to the subject with the chairmanship of the presidents of the Royal College of Physicians of London and the Royal College of Pathologists.

In his introductory lecture, **Professor Lambert** (St George's Hospital, London) suggested three areas of change that deserve particular interest. First, he pointed to the recent studies of the pathogenesis of bacterial meningitis which have emerged largely from the United States. It has become clear that, while bacterial invasion and the release of cell-wall products, such as endotoxin, remain important, much of the damage that occurs in bacterial meningitis results from the elaboration of inflammatory cells, cytokines and other inflammatory mediators. Interestingly, several groups have observed that administration of antibiotics is associated with a rise in endotoxin concentrations in the cerebrospinal fluid, resulting in a marked increase in the intensity of the destructive inflammatory process. This observation is most striking with highly bac-

tericidal antibiotics such as the cephalosporins and has led to the widely held belief that other adjunctive therapies are required if the damage associated with bacterial meningitis is to be prevented.

Second, Professor Lambert considered the new developments in the field of bacterial adaptation to the environment. He explained that the interaction of a regulator gene and unlinked virulence genes is of primary importance in determining bacterial behaviour in response to environmental stimuli. The cholera tox R gene, for example, has been shown to regulate toxin and pilus production in response to local osmolarity and amino acid concentrations. This clearly has important implications for our understanding of bacterial behaviour *in vivo* and *in vitro*.

Third, he pointed to the impact of new technology on bacterial epidemiology. Recombinant techniques have revealed the limited clonality of specific organisms such as invasive *H. influenzae* type b, and have improved our ability to study the mechanisms of epidemics. This is illustrated by a study of the meningococcal outbreak among pilgrims returning from Mecca, which linked as many as 11% of cases to one single meningococcal clone. These techniques have allowed us to study, in detail, the new diseases that have arisen from well-described, previously benign organisms. Brazilian purpuric fever, for example, is thought to have resulted from the incorporation of a 25-megadalton plasmid into the DNA of *Haemophilus aegypticus*.

Dr Miles (London School of Hygiene and Tropical Medicine) and **Dr Quint** (SSDZ, The Netherlands) continued this fascinating theme with a discussion of the value of DNA recombinant techniques in diagnosis and management. Dr Miles revealed that a specific probe has been developed to the *Leishmania donovani complex*, which can detect the organism in species as diverse as flies, hamsters and humans. Employing the polymerase chain reaction (PCR) it is now possible to screen for dog reservoirs, and it will not be long before *leishmania* will be detectable in the field in human leukocytes. The knowledge of specific *leishmania* genes may also be applied to manufacture recombinant proteins for use as diagnostic reagents and candidate vaccines. Contamination with foreign DNA is becoming less of a problem, and with the development of non-radioactive techniques Dr Miles sees PCR as an increasingly valuable tool. Malaria, for example, may be detected even at a time when a blood film is negative, and PCR should be able to identify drug resistance genes, so guiding therapy, and the development of prophylactic measures.

Dr Quint also emphasised the sensitivity and flexibility of PCR. His group has employed this technology to identify the human papilloma virus (HPV) in cervical scrapings, paraffin embedded tissue, and urine. They have also exploited PCR to locate genital *chlamydia* in cases where the cultures are negative. PCR can be used to detect several organisms simultaneously, and Dr Quint is currently exploring the epidemiology of HPV and *chlamydia* infections.

Professor Durack (Duke University, USA) challenged the assertion by William Osler that little more is to be known about infective endocarditis. Since Osler's time, the patient population has become older, chronic rheumatic fever less common, and right sided endocarditis in drug addicts and valve infection with coliforms and fungi have become significant problems. In the 1990s, mitral valve prolapse and the prospect of home therapy for endocarditis with once-daily injections of ceftriaxone have become an important topic. It is now clear that the disease may recur even after many years and that recurrences are more common than originally suspected. Similarly, although the incidence of early-onset endocarditis on prosthetic valves is falling, the incidence of late disease remains worryingly high. Professor Durack also pointed out that, in step with the changing face of endocarditis, the variety and sensitivity of diagnostic imaging techniques have improved significantly over the past few years. In experienced hands, transoesophageal echocardiography, for example, is able to detect over 90% of cases.

The afternoon session concentrated on tropical medicine and illustrated that a complete understanding of such well-known diseases as malaria and TB remains elusive. **Professor Luzzatto** (Royal Postgraduate Medical School) discussed the impact of the haemoglobinopathies on the epidemiology and clinical features of malaria. It has now been well established from α-thalassaemia gene studies, and analysis of the relationship of genotype to malaria parasitaemia, that the haemoglobinopathies influence the geographic distribution of malaria. What is not certain, however, is the precise mechanism of protection. In sickle trait individuals, a number of mechanisms have been proposed: parasite destruction by HbS polymers, increased disposal of cells that sickle following schizogony, and increased phagocytosis of HbAS cells. It has also been suggested that a delay in parasite maturation and defective schizogony may occur in association with glucose-6-phosphate deficiency.

Professor Warrell (John Radcliffe Hospital, Oxford) considered the areas in which an understanding of the pathophysiology of cerebral malaria might lead to improved therapeutic interventions. While the exact cause of the cerebral syndrome remains controversial, many believe that adherence of parasite-laden erythrocytes to the vascular endothelium and their subsequent sequestration within the brain is important. Recently, a specific adhesion molecule, *Plasmodium falciparium* erythrocyte membrane protein, has been identified on infected erythrocytes. It binds to the specific endothelial surface molecules ICAM-1, CD36, and thrombospondin. Blocking antibodies and receptor analogues are therefore being developed to prevent cytoadherence and sequestration.

Professor Warrell also expanded on the role of

cytokines in the pathogenesis of cerebral malaria and pointed to the relationship between high levels of tumour necrosis factor (TNF) and mortality from the disease. He suggested that this may, in part, be related to the observation that IL-1 and TNF increase endothelial binding of phagocytosed erythrocytes in experimental models. Intervention studies with anticytokine antibodies are already in progress, but Professor Warrell emphasised that clinical physiological studies performed on non-immune adults in Thailand and on children from hyperendemic Africa are yielding very different results. This suggests that host differences may be important in determining the pathophysiological picture of the disease, and may determine appropriate therapeutic intervention.

Professor McAdam (London School of Hygiene and Tropical Medicine) disclosed the rather depressing picture of the resurgence of tuberculosis in association with human immunodeficiency virus (HIV). This has occurred particularly in Africa where HIV seropositivity ranges from 9% to 60%. The interaction between TB and HIV is not clear, but it seems that the retrovirus not only increases reactivation of TB but may also enhance susceptibility. Characteristically, these patients are less likely to be sputum positive, there is less cavitation, and more nodal disease. Standard drug therapy is employed but adverse drug reactions are more common. The issue of maintenance therapy following completion of treatment remains unanswered. Equally, while in the United States many have adopted primary prophylaxis, it is questionable whether it would be practical or economic to adopt this policy in developing countries. Indeed, we are just beginning to appreciate the devastating effect HIV is having on such communities, and the enormous economic impact of the resurgence of TB on both individuals and health services.

Professor Bradley (London School of Hygiene and Tropical Medicine) discussed the development of animal models for tropical diseases. There has been considerable development since the early work of Smith and Kilborn on Southern cattle fever, Manson on malaria, and Reed on yellow fever. Several of the models which have been developed to emulate human disorders are providing useful clues to the pathogenesis of diseases such as malaria and hepatitis B. Isolated animal organs play a vital role in the development of bio-assays, and inbred mice are used as important models for the investigation of the effects of specific genes on infection. While cell culture has largely replaced animal culture, the latter still has a place in detecting leprosy, for example.

The first day of the conference was well received by the audience, and many of the presentations provoked lively discussion. These sessions emphasised the rapidly changing nature of the specialty and the need for a multidisciplinary approach to the subject.

Second day

The genetic basis for susceptibility to infection and disease was summarised by **Dr A. V. S. Hill** (John Radcliffe Hospital, Oxford). He reviewed recent studies on malaria and HLA and related them to his own work on the structure of the relevant HLA binding site. Although twin studies show an important genetic contribution to disease in leprosy, tuberculosis and poliomyelitis, the paucity of HLA associations does not make it obvious which genes might be responsible. Malaria is a good model for study since the AS genotype affords 90% protection against severe malaria. Recent evidence links HLA Bw53 and DR1302/DQ1 respectively with protection against severe malaria and severe malarial anaemia. An elegant series of experiments has revealed the structure of the host site which binds antigen. The next step is to discover against which malarial antigen this structure mediates its protective effect.

Dr J. Bell (John Radcliffe Hospital, Oxford) extended the discussion of genetic susceptibility to autoimmune disease. T cell activation plays a part both in infectious disease and in autoimmunity. In infectious disease such activation is a mechanism for producing protection; in autoimmune disease it produces tissue destruction. Foreign antigens are presented to T helper cells in association with class II molecules and the class II antigens DP, DQ, and DR have the strongest associations with autoimmune disease. Polymorphisms in these antigens are found at the contact points in the alpha helices and on the beta sheet at the floor of the groove where foreign antigen is bound.

John Bell went on to describe three autoimmune diseases with a putative structural basis for susceptibility:

i Type I diabetes is associated with both class I and class II antigens, but DQ appears to be the major susceptibility molecule. Amino acid changes at position 57 of the β chain correlate with susceptibility to diabetes. Neutral amino acids at this position are associated with susceptibility while aspartic acid protects.

ii In rheumatoid arthritis the DR4 and DR1 associations share an allele, a single arginine/lysine switch. Here the presence of a charged amino acid at the site in the molecule where the antigen is likely to sit is associated with decreased susceptibility.

iii The association of susceptibility to coeliac disease with several D antigens is explained by the sharing of a DQ molecule.

Dr C. C. Blackwell (University of Edinburgh) related secretor status to susceptibility to infectious agents. Secretion of the major blood group glycoprotein antigens in body fluids is controlled by a gene coding for a fucosyl transferase acting in secretory tissues. Secretion is genetically dominant in Caucasian populations. Infections with *Streptococcus pyogenes, Neisseria meningi-*

tidis, Streptococcus pneumoniae, Haemophilus influenzae, Vibrio cholerae and *Helicobacter pylori* (in duodenal ulcers) and caries are more common in non-secretors. Studies to elucidate the mechanism for this association have examined Lewis or H antigens as the putative molecules. Experimental results are consistent either with Lewis *a* antigen receptors acting as an attachment site for micro-organisms on some host cells, or with ABO or Lewis *b* antigens in the secretions of secretors binding to microbial adhesins to reduce colonisation.

Dr G. E. Griffin (St George's Hospital, London) described how the Human Immunodeficiency Virus produced the changes in immunity for which it is especially noted. He made the point that the term 'asymptomatic HIV infection' is a misnomer now that subtle changes short of full-blown immune deficiency, such as partial villous atrophy, can be measured. He reviewed the types of cells known to be targets for HIV, mentioning new work on eosinophils. There is a panoply of effects on the humoral, cellular and secretory immune systems. Polyclonal B cell activation is defective. Patients with persistent generalised lymphadenopathy have a relatively large number of specific cytotoxic lymphocytes which decrease as disease progresses, allowing control over virus replication to escape. The effects associated with low numbers of CD4 cells may indicate the responsibility which these cells have for surveillance of latent infections. HIV infected patients have decreased numbers of IgA plasma cells, but no correlation exists between enteric infection and IgA levels. However, these patients do have decreased IgA and IgG and absent IgM serum responses to giardia. HIV infection does not change the numbers, phenotype or function of peripheral blood macrophages but some HIV strains are 1,000-fold more tropic for these cells. Virus assembles in vesicles, producing a slow release and turnover. Activation of macrophages, however, results in enhancement of HIV transcription and the release of cytokines, which themselves are responsible for weight loss and fever. Soluble gp120 molecules can switch on macrophages to release cytokine. IL6 is produced in the later stages of HIV infection and stimulates B cells.

Dr G. C. Schild (National Institute of Biological Standards and Control, London) explained how new developments in biotechnology offer promise in the design of new vaccines. In contrast to the killed vaccines of yesterday, non-replicated antigens can be synthesised by expressing rDNA in bacteria, yeast or mammalian cells, producing proteins that are glycosylated and folded like the native proteins. Entirely synthetic vaccines in the form of synthetic oligopeptides also hold promise, although there are a number of caveats. Antigenic sites are not always composed of contiguous amino acid sequences; more than a single epitope might be needed to produce a successful antigen; antigenic sites could be altered by virus mutation; and peptide antigens seem to produce poor cytotoxic lymphocyte responses. A third strategy resides in anti-idiotype technology, where an immunoglobulin itself becomes an antigen. Genetic techniques can also be used in the development of new live vaccines. A virulent virus might be attenuated by precise genetic modification when its gene function becomes understood. Poliovirus has been studied most intensively and the sites responsible for neutralising antibody responses and for various properties of the virus have been identified. Genes for specific proteins can be incorporated in a large virus such as vaccinia and expressed during virus replication in the host, producing an antibody response. This strategy has been successfully employed for rabies, yielding a vaccine that has been used in foxes. But despite the promise implicit in the new methods, only a single new vaccine, that for hepatitis B, has so far reached the market.

Dr Schild went on to describe the challenges to science posed by a vaccine against infection with the Human Immunodeficiency Virus. Either inactivated or live attenuated virus vaccines of the traditional type are ineligible candidates because residual, intact nucleic acid in tandem with the known genetic instability of the virus produce too great a risk that the vaccine would induce infection. Study of the antigenic sites on the virus has been greatly facilitated by biotechnological approaches, but use of single peptide antigens, though immunogenic, has consistently failed to provide protection against challenge infection in chimpanzees.

Dr P. A. Kitchen (National Institute of Biological Standards and Control, London) detailed the current status of candidate HIV vaccines. HIV is difficult to work with because chimpanzees are the only animal model, but a model for infection only, as their HIV infection does not produce immunodeficiency. This disadvantage, combined with the failure to induce protective immunity using recombinant antigens, has caused efforts to be directed into new avenues. Rather than working with HIV directly, experiments have been performed with simian immunodeficiency virus (SIV). Many parallels exist between HIV and SIV and there is good reason to believe that what has been learned from one could be applied to the other. Several workers have now shown that a killed, whole virus vaccine given with an adjuvant successfully protects Rhesus monkeys against infection with homologous strains of virus. Work is now proceeding on more efficient vaccination schedules and on defining the range of strains against which protection is afforded. Although promising, these results still have some drawbacks. Protection appears to be short-lived, its duration being limited to four months following the last booster. Despite initial hopes, post-exposure vaccination has also failed to protect against disease.

Dr David Tyrrell chaired the session on training in infectious diseases. It featured short presentations on infectious disease training from the viewpoint of a US physician, a UK physician/microbiologist and a UK microbiologist. **Professor David Durack** presented an

account of how infectious diseases has burgeoned as a clinical specialty in the United States, highlighting the growth in membership of the Infectious Disease Society of America from around 1,000 members in 1980, comprising individuals from academic institutions, research and industry, to around 3,000 members in 1990, half of whom were in private practice in infectious disease. Training in infectious disease is well organised, usually including time for research, but only sometimes for microbiology. **Professor Roger Finch** described the more variable arrangements for clinical infectious disease training in the UK, the paths to specialist status traversing adult infectious disease, tropical medicine, medical microbiology, public health, paediatric infectious disease and research. The minuscule numbers of UK clinicians dedicated to infectious disease practice as compared to the US was explained by **Professor Ian Phillips's** defence of the traditional role played by medical microbiologists, who combine with general physicians in many hospitals to provide a service for infectious diseases.

During the discussion reference was made to the report of a joint working party of the Royal College of Physicians and the Royal College of Pathologists on training in infectious diseases, published in July 1990. It has been agreed to set up a standing committee to monitor future developments; one of its early tasks will be to address the problem of training in paediatric infectious diseases. The report of the working party depends heavily on guidance and funding from the Department of Health for the implementation of its recommendations. It is difficult to predict their fate in a market-led health service, where funding follows patients and where clinical activity plays a larger role than central decision making in determining hospital income. The balance between medical microbiology and clinical infectious disease in the UK may in the end be determined by factors of cost effectiveness and income generation rather than the opinions of the wise.

Report of first day
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Report of second day
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