Sir William Leishman

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WILLIAM Boog Leishman, whose memory we are honouring this evening, was born 100 years ago into a family which in the immediately preceding generations had achieved considerable distinction in clerical and medical circles in the West of Scotland. His grandfather, the Rev. Matthew Leishman, D.D., was well known as the Minister of the Parish of Govan in Glasgow, and ultimately became Moderator of the Church of Scotland, while at a later date an equally eminent uncle, the Rev. Thomas Leishman, D.D., occupied the same high office, a father-and-son record which is probably unique. His father, William Leishman, graduated in medicine, and from 1868 to 1893 was Professor of Midwifery and also for a number of years Dean of the Faculty of Medicine in the University of Glasgow. Our Director-General-to-be was sent to England for his early education, first to a preparatory school in Kent and later to Westminster. He returned to Glasgow to study medicine and qualified in 1886 at the early age of 21. Without filling any hospital appointments, he applied for and was granted a commission as Surgeon on the Medical Staff of the Army (at that time the Corps as such was not in existence). A few years of home service were followed by a tour of duty in India, where he saw active service in the Waziristan Expedition, 1894-95. It is on record that during this period in India he carried with him his own personal microscope, presumably to examine blood smears for the recently described malaria parasites. Returning home in 1897, he was posted to the Army Medical School, then housed in the Military Hospital at Netley, and was appointed Assistant Professor of Pathology. The Professor was a civilian, no less a person than Almroth Wright, later to achieve fame for his studies in immunology. In 1902 the Army Medical School left Netley Hospital and the Departments of Hygiene and Pathology moved to temporary quarters in the laboratories of the Royal Colleges of Surgeons and Physicians in London. In 1903 Wright resigned his Chair to go to St. Mary's Hospital, and Leishman was appointed Professor in his stead. In 1907, when the building in which we are now meeting was completed, he moved with his staff into the laboratories adjoining this lecture theatre, where he continued to teach and pursue his researches for the next 6 years. In 1913 this phase of his career came to an end, when he was transferred to the War Office as Expert in Tropical Diseases to the Army Medical Advisory Board. This appointment was interrupted by the outbreak of war, and in 1915 he went to France as Adviser in Pathology to the Director of Medical Services. Returning to the War Office in 1919, he became the first Director of Pathology and in 1923, with the rank of Lieut-General, succeeded to the office of Director General of the Army Medical Services. He died in 1926 while still holding this appointment.

Leishman received many honours and awards. In 1905 he was made Brevet Lieutenant-Colonel in recognition of his distinguished researches. He was created Knight Bachelor in 1909, and elected F.R.S. in 1910. In 1912 he was appointed K.H.P. and during the 1914-18 War he was thrice mentioned in despatches, and was awarded the C.B., the Legion of Honour (3rd Class) and the American Distinguished Service Medal.

When Director of Pathology he was made K.C.M.G., and when Director General, K.C.B., Grand Officer of the Legion of Honour, and Knight of Grace of the Order of St. John of Jerusalem.

He was chosen as one of the original members of the Medical Research Council when this body was constituted in 1913 and was elected F.R.C.P. in 1914.

From this brief *curriculum vitae* we may now turn to a more leisurely consideration of Leishman's achievements.

Leishman's Stain

One of Leishman's earliest and best known discoveries was the stain which bears his name. In skilled hands it is unsurpassed for staining blood-smears, and is particularly useful for bringing out the details of malaria parasites, trypanosomes and other blood parasites. Its special property is that it stains the chromatin or nuclei of these organisms a distinctive reddish purple colour which is different from the colour of the nuclei of the leucocytes and renders the parasite conspicuous and easy to identify. The underlying principle of this stain was discovered at an earlier date by Romanowsky[1], who showed that a mixture of solutions of ripened methylene blue and eosin had this particular staining property, and found that this was attributable to the formation of a third component, methylene azure, consisting of Azures A and B. In 1899 Jenner, using the Romanowsky principle, produced a solid stain, soluble in methyl alcohol, which proved useful for haematological work, but not for the detection of blood parasites. Stimulated by results described by Dr. Georg Maurer of Sumatra, Leishman experimented with the modified Romanowsky technique he recommended, and was sufficiently satisfied with his results to publish details in the British Medical Journal early in 1901. Briefly, he fixed blood smears in alcohol and ether, dried them off and then pipetted on to the dry smear equal quantities of polychrome methylene blue and eosin, ensuring that mixing occurred just as the solutions came in contact with the smear. Staining was allowed to continue for ½ to 1 hour, after which the slide was washed and the smear decolourised either by alcohol, or, more slowly, by distilled water. Excellent results were obtained in this way, but the process was laborious. Leishman noticed that, as the two stains mixed, a scum formed and precipitated on to the slide, and further found that when bulk quantities of the two stains were intermingled, a precipitate settled out which could be filtered off and dried. This precipitate, which is the substance known as Leishman's stain, is soluble in methyl alcohol, and has the same properties as the newly mixed watery stains. Everyone is familiar with the method of using this stain, but nevertheless a cautionary remark is necessary. Leishman was both artist and perfectionist. The details of the staining technique, given in his second paper in 1901, were not arrived at by chance, but were the result of many careful controlled experiments. His instructions should therefore be followed with meticulous care. It is perhaps worthy of mention that the two further modifications of Romanowsky's stain which are in common use—Wright's, differing from Leishman's only in the method of ' ripening ' the methylene blue, and Giemsa's, a more stable preparation which however lacks the convenience of Leishman's stain—were described in 1902, a year after Leishman's publication.

Antityphoid Vaccine

When Leishman took up his duties in Netley in 1897, he immediately became involved in the study of active immunisation against typhoid fever, a subject which was to remain throughout his life one of his dominant interests. Some years earlier his chief, Almroth Wright, had made contact with Haffkine, then on the staff of the Pasteur Institute in Paris, where he was engaged in exploring the possibilities of vaccination against cholera. Wright was quick to realise that a similar procedure might be of use in the prophylaxis of typhoid fever, an infection then of common occurrence in Britain as well as overseas, and at once began a series of experiments and trials. His first paper, describing the early and crude techniques followed in making the vaccine, and a few preliminary trials, was published about the time Leishman joined his staff[2]. In the next few years, a more satisfactory but still crude vaccine was evolved. This was used in a limited way in an asylum in Kent where there was an outbreak of typhoid fever, and also in some regiments in India. The results appeared favourable, but there were no controls of any kind to give the figures statistical validity. The South African War provided an opportunity for a more extensive trial, and with some reluctance authority was given for the troops to be inoculated, on a voluntary basis, with vaccine which Wright supplied. The response, however, was very half-hearted, and indeed because of the severe reactions which the vaccine produced inoculation was very unpopular, so that in all not more than about 4% of the men were protected in this way. Under active service conditions it was impossible to obtain reliable data about the subsequent medical history of the men who had been inoculated, and the only figures of any value came from the garrison of besieged Ladysmith, where the ratio of incidence in the non-inoculated majority was 1 in 7.07, while in those who had been inoculated was 1 in 48.7. Though these figures appeared reasonably encouraging, the statisticians refused to accept them as evidence of the value of inoculation, and a violent onslaught on the conclusions which Almroth Wright formulated, headed by no less a person than Karl Pearson, led to the withdrawal of permission to inoculate Army personnel and, less directly, to the resignation of Wright from the Chair of Pathology.

During all this time, while Wright was in the limelight, Leishman was busy in the background, devising better methods of preparing the vaccine, and of assessing its potency. Though convinced that it was of value as a prophylactic, he was dissatisfied with the vaccine used in the South African War, and believed that with further research it would be possible to produce a more reliable and effective preparation.

Some time after Wright's resignation and in response to continued agitation for which he was mainly responsible, the War Office reopened the question, and in 1904 appointed an Antityphoid Committee with a list of distinguished members, including among others, C. J. Martin, who became Chairman, Sir David Bruce, Almroth Wright himself (who however resigned after a few months) and Leishman. The Committee, after due deliberation, nominated Leishman to carry out such investigations as he thought advisable, and to this end recommended that he should have all necessary facilities and be authorised to co-opt assistants from among the junior officers of the Corps.

The researches of the next few years are a model of the way in which an investigation of this kind should be conducted. Experiments were carried out under no fewer than thirty headings, most of which called for repeated tests, while each heading led in a logical way to the next in the series. A preliminary step was the establishment of methods for evaluating the efficacy and potency of batches of vaccine prepared in different ways. With unimportant exceptions, the experimental animal throughout the investigation was the human volunteer. In most experiments several volunteers were inoculated with the particular modification of the vaccine which was under trial, and pooled serum from these men was used in the subsequent tests. The sera were tested for agglutination titre, for bactericidal and bacteriolytic properties, which Leishman came to regard as successive stages of the same process, and for opsonins and stimulins, which he also decided were different aspects of the same phenomenon and which in his later work he referred to as phagocytic properties or phagocytic activity. Based on his experiments he came to look on phagocytic action as the most important index of immunity, but nevertheless regarded the development of agglutinins as a reasonable guide. In parenthesis, it may be mentioned that he found no evidence of the so-called negative phase—a conception of Wright's which his opponents used as an argument against mass immunisation. Many different methods of sterilisation and several different "antiseptics" were examined. It was found that heating the vaccine to 65° destroyed some of its immunising properties and that 1 hour at 53°C. was optimum. As an antiseptic and preservative, 0.4% lysol gave the most favourable results, but it proved necessary to wait until the vaccine had cooled before adding the lysol, otherwise it had a deleterious action. Of the various chemical sterilising agents tested, it is interesting, in the light of more recent work, to note that alcohol was one. This proved to be effective in killing the bacteria, but vaccine sterilised in this way was not tested for its immunising properties as it was noted that at blood temperature bacteria treated with alcohol did not autolyse, and Leishman argued that, as the organisms were unlikely to be readily broken up when injected into the body, an alcohol-killed vaccine would probably be inefficient in stimulating the development of anti-bacterial substance.

To test the keeping properties of the vaccine, a batch was sent for a voyage round the world. On its return a year later it had lost much of its immunising properties. Even when stored in the laboratory at room temperature, vaccine was found to have undergone considerable deterioration in 6 months. The necessity for cool storage became obvious.

Tests were made to ascertain if a vaccine made from a virulent strain of the typhoid bacillus was more effective than one from the relatively non-virulent strain which had been selected because of its good antigenic properties, but no difference could be detected. Later experiments showed that inoculation with a vaccine prepared from this non-virulent strain produced bactericidal substances active against other and more virulent heterologous strains.

The conditions necessary for optimum bacterial growth were investigated, and Leishman finally selected culture in nutrient broth with a reaction of + 10 on Eyre's scale—a method of standardising reaction which has long fallen into disuse. The figure quoted—+10—is approximately pH7. To ensure adequate aeration the medium was prepared in Roux bottles which after inoculation were placed on their sides in the incubator, thus giving a shallow layer of medium with a large surface exposed to atmospheric oxygen.

The number of bacteria per c.c. of vaccine was calculated by mixing a suitable dilution of the bacterial suspension with an equal quantity of normal blood which was assumed to contain five million erythrocytes per cubic millimetre. The bacteria and red cells in a number of microscopic fields were then counted, and from these figures the number of bacteria per c.c. was calculated. Wright's original method, using untreated blood, was found to give irregular results partly because the serum tended to clump the bacteria, and possibly because of variable bacteriolytic activity. This difficulty was overcome by using washed red cells suspended either in broth or saline sufficient to give a volume equal to the original volume of the blood sample.

Further experiments showed that, while a single dose of vaccine resulted in the production of antibodies, a much higher level was achieved if 2 doses were given at an interval of 10 days. The optimum bacterial content of the 2 doses was found by trial to be 500 million and 1,000 million respectively. In an attempt to cut down the reaction which followed inoculation, washed bacteria in saline were substituted for the whole broth culture, but this made no significant difference. Besredka's method of giving combined vaccine and antiserum was explored but found to have no advantage. It was demonstrated that there was less local reaction if the vaccine was given subcutaneously, that the best site for injection was over the insertion of the deltoid, that the best time of day for inoculation was the late afternoon and that it was imperative to avoid alcohol for 48 hours after being inoculated. Finally the possibility of giving the vaccine by mouth was investigated. Bacteria, in a series of graded doses, were incorporated in glycerine or fat and administered in capsules. Some antibody production resulted, but this was too irregular and uncertain for the method to be of practical value.

All these points, which we of our generation are apt to take for granted and perhaps even regard as empirical, were established by careful experiments. Leishman's work is in fact the foundation stone of vaccine manufacture in the Army.

As a sort of postscript, the influence of typhoid immunisation on paratyphoid infection was investigated. No appreciable degree of immunity was found.

However convincing the results of these laboratory experiments might be, their validity had to be verified by a field trial, and this also was organised by Leishman. From the end of 1904 until the beginning of 1909, the period during which this trial was in operation, he arranged that each regiment going on overseas service—whether infantry, cavalry or gunner—should have a medical officer attached to it permanently for 3 years. Before joining his regiment each of these officers was given, under Leishman's guidance, a special course of instruction on the principles and techniques of inoculation, and on the clinical and laboratory diagnosis of typhoid fever. He was also given details of the records he must keep of all the men in the regiment, whether inoculated or not (for inoculation was on a voluntary basis and a 50% response was anticipated) and was impressed with the necessity for a careful follow-up of such men as were transferred from the regiment. For reasons obvious to anyone familiar with service conditions, many difficulties were encountered in carrying out these instructions to the letter. Nevertheless, the final returns were believed to be reasonably accurate. In all, 24 units were included in the trial, and the average period of observation of each was 1 year 8 months. The total number of individuals, warrant officers, non-commissioned officers and men, was 19,314. Of these, 10,378 were inoculated and among them there were 56 cases of typhoid fever, of whom 5 died. 8,936 were not inoculated among whom were 272 cases and 46 deaths. The case incidence per 1,000 was therefore 5.39 and 30.4 respectively, and the mortality percentage 8.9 and 16.9.

These experiments and findings were embodied in a brilliant series of papers published by Leishman in the Corps Journal, were summarised in the Harben Lectures, which he delivered before the Royal Institute of Public Health in 1910, and finally appeared officially in the Report of the Antityphoid Committee in 1912. Of this Report, published under a list of distinguished names, it may be said, (to paraphrase Isaac's words when giving his blessing to one of his sons) that while the hands were the hands of the Antityphoid Committee, the voice was the voice of Leishman.

Whoever may have thought otherwise, Leishman himself had no doubts about the efficacy of antityphoid inoculation in preventing typhoid infection, and in the ensuing years he published papers recording these findings, and the conclusions to be drawn from them, in a wide range of medical journals, and did everything in his power to convince the authorities and the public of the value and importance of this procedure. Despite his efforts, inoculation in the British Army was not made compulsory, but during the 1914-18 war, in his capacity as Advisor in Pathology to the Director of Medical Services in France, he was able to bring strong pressure to bear, and as a result the inoculation state of the troops was commendably high. Because of the different environmental conditions in the South African War and the 1914-18 War, no valid comparison can be made between the figures of the two campaigns, but it is perhaps worth recording that in South Africa the annual incidence of typhoid fever per 1000 of strength was 105, while in the 1914-18 War it was 2·35. The sections in the Official History of the First War on typhoid fever and antityphoid inoculation come from Leishman's pen.

At this point Leishman's personal association with antityphoid inoculation came to an end, but in view of subsequent happenings and criticisms it is only just to his memory to follow the subject to what may perhaps be its logical conclusion. In the interwar years the strain of typhoid bacillus selected by Leishman for making the vaccine was found to have undergone some degradation, but its virulence was restored by mouse passage, and in this ' rejuvenated ' form it continued to be used for vaccine manufacture. During World War II the inoculation state of the British troops was good, and despite epidemics of typhoid fever among prisoners of war before they could be inoculated with our Army vaccine, and later among the civilians of Middle East, particularly in Palestine, the incidence of this disease among British troops was negligible. In the absence of controls no statistical evidence of the value of inoculation can be produced, but there were many straws to show how the wind was blowing, and no unbiassed observer had any doubts on the subject. About this time strong claims were being made regarding the virtues of an alcoholised vaccine, which was richer than phenolised vaccine in so-called Vi antigen and which was more effective in protecting laboratory animals against intraperitoneal challenge with virulent typhoid bacilli. In 1943 it was decided to stop making phenolised vaccine, and to replace it, as stocks became exhausted, with alcoholised vaccine. At the same time a further change was made. Experiments in laboratory animals seemed to indicate that the number of bacteria per dose was unnecessarily high and so, with a view to reducing the local and general reactions which follow inoculation, the strength of the vaccine was cut down by half. A year or two later sharp outbreaks of typhoid fever began to occur among British troops in the Middle East Force, outbreaks which defied the most stringent hygienic precautions, and it became very clear either that the challenge was abnormal or that the vaccine was lacking in protective properties. It was argued that if the vaccine was at fault this might be attributable either to the different method of preparation or to its reduced strength and an attempt was made to solve this problem by giving to alternate subjects phenolised and alcoholised vaccine respectively, but with the alcoholised vaccine restored to the strength recommended by Leishman. In one sense the experiment was a failure, as under the existing conditions of service it proved impossible to ensure that each man received the correct vaccine when his second dose fell due. However, coincident with the mounting of this experiment, the outbreaks of typhoid fever ceased and the incidence reverted to its previous low level. For various reasons it was decided in 1954 to revert to phenolised vaccine, and since then no further incidents have occurred.

This unfortunate experience stimulated sceptical statisticians to raise their voices once again, and doubts were cast on the validity of Leishman's conclusions. It is obvious that Leishman had anticipated such criticism, for the penultimate paragraph of the 1912 Report runs as follows: " Leaving on one side the statistical validity of the figures, it will be admitted that it is not easy to explain the facts that in the smaller group of 8,936 non-inoculated men 46 died from enteric fever, while in the larger group of 10,378 men there were but 5 deaths from this disease, on any other grounds than the protection afforded by inoculation ".

However, recent field trials in different parts of the world have provided evidence of the efficacy of typhoid vaccine which stands up to the most searching criticism[6]. The last of these is perhaps the most convincing. At the request of the Medical Officer of Health in British Guiana, where typhoid fever is rife, especially among school children, the Colonial Medical Research Committee (as it was called at that time) with co-operation from the World Health Organization, United States Army Medical Centre and certain other interested parties, agreed to organise a controlled campaign of mass inoculation. Three vaccines were prepared, one a phenolised typhoid vaccine, the second an acetone-killed typhoid vaccine, and the third tetanus toxoid. These were bottled and packaged in such a way as to be indistinguishable one from the other except by the code letter on the label, and none of those who carried out the trial had the key to this code. The team conducting the trial, employed on a whole-time basis, consisted of an experienced research worker (epidemiologist), a recently retired Public Health Service bacteriologist, and technical staff. On a part-time basis nursing sisters and health visitors to assist in inoculation and supervision were detailed by the Medical Officer of Health in British Guiana. Three groups of school children between the ages of 5 and 15, each group numbering around 23,000 and each child selected in a random fashion, were inoculated with 2 doses of one or other of the 3 vaccines. A very careful follow-up was maintained for 2 years, and was in fact carried on after that date. Every child suffering from fever of any kind was reported by the school teachers, examined by the epidemiologist, and if necessary admitted to hospital where laboratory tests were carried out. A diagnosis of typhoid fever was made only in cases which gave a positive blood-culture, or, occasionally in patients showing characteristic clinical symptoms from whose faeces the typhoid bacillus was isolated. The ' score ' at the end of 2 years (the arbitrary period during which typhoid vaccine is expected to give protection) was:- tetanus toxoid group, 70 cases; phenolised vaccine group, 17 cases; acetone-killed vaccine group, 5 cases. Thus the phenolised vaccine, assessed against the tetanus toxoid control, gave approximately 75% protection, a figure not far different from that obtained by Leishman in his field trial. His conclusions are therefore amply confirmed, and his faith in the value of anti-typhoid inoculation fully justified. The striking results in the group inoculated with acetone-killed vaccine hold out promise of further progress.

Kala-Azar

In 1903 Leishman published a paper entitled " On the possibility of the occurrence of trypanosomiasis in India " which was the opening gambit of a long series of observations and papers culminating in the identification of the complex of diseases grouped together under the eponymous name of Leishmaniasis. The story, however, begins at an earlier date. In 1900 a soldier who had been stationed in Dum-dum, an unhealthy garrison some 7 miles from Calcutta, was invalided home suffering from a long-standing fever, and was admitted to Netley Hospital. Fevers such as this, characterised by chronicity, by grave anaemia, by progressive muscular atrophy and by a grossly enlarged spleen, were of common occurrence in Eastern Bengal, and for want of a more specific diagnosis were called Dum-dum fever. The patient died 7 months after reaching Netley, and was autopsied by Leishman, who among other investigations made and stained smears from his spleen. These were found to contain large numbers of small oat-shaped bodies measuring up to 5μ in their long axis, which occurred either within the macrophages or lying free between the cells. Leishman had no doubt that these were protozoal parasites but he had never before seen bodies of this kind, and with characteristic caution he deferred publication of his findings until some confirmatory evidence was forthcoming. Two years later, in the course of some experiments with trypanosomes of the *brucei* group, he made blood smears from an infected rat which had been dead for 2 days, and in them found both trypanosomes of normal appearance and degenerated forms which closely resembled the bodies he had encountered in the spleen smears of the patient who died of Dum-dum fever. This led him to believe that the Dum-dum parasites might also be degenerating trypanosomes, and encouraged him to publish these earlier findings in the British Medical Journal. A few months later (in fact, in the time taken for the British Medical Journal to reach India and a comment on Leishman's paper to be sent home) a letter from Dr. C. Donovan[4] appeared in that journal stating that in April 1903, and again in June, he had found bodies similar to those described by Leishman in smears made from spleen punctures of patients suffering from prolonged fever similar to Dum-dum fever. This observation ruled out Leishman's theory that the parasites were trypanosomes undergoing post-mortem digestion. The issue was further complicated by a report from Wright[5] that he had found similar bodies in the pus of a tropical ulcer (Delhi sore). Donovan meantime sent the spleen smears from his cases to Ronald Ross, who confirmed Donovan's findings, but gave it as his opinion that the parasites were not trypanosomes. Ross also remarked that the charts and records of Donovan's cases were a little suggestive of kala-azar. Leishman, replying to Ross, disagreed with his opinion on the nature of the parasites. He still considered that there might be some connection between these bodies and trypanosomes, and went so far as to suggest that the life-history of some trypanosomes might include a stage of encystment. Meantime another authority in the person of Laveran[7]—the discoverer of the malaria parasite—entered the discussion and suggested that Leishman's bodies were *Piroplasmata*, related to the parasite which caused redwater fever in cattle. Ross[8] dissented from this hypothesis and propounded the theory that the parasites were a species of *Sporozoa*. He proposed that they should be named *Leishmania donovani*. Leishman however, stuck to his guns. He too put aside Laveran's idea that the parasites were piroplasms. He recalled Wright's description of the parasites in " Delhi sore ", and also that he had at one time been informed by Ross that the latter had frequently found in the pus of Delhi sores large numbers of the flagellate *Cercomonas hominis*. He suggested that Wright's parasites might be altered cercomonads, and made the shrewd proposition that a study of these cercomonads might throw light on certain aspects of the problem. Yet another personality now began to take an interest in the subject. In 1896 Leonard Rogers had been sent to Assam to investigate an epidemic of kala-azar which was sweeping slowly through that country. He established the important fact that the striking range of the disease was very short, and that the epidemic could be halted locally by moving coolies from infected quarters to clean huts 200 yards away, but he failed to discover the causative organism, though in a tentative and half-hearted way he blamed the malaria parasite. Following the correspondence sparked off by Leishman's discovery, he saw a possible association between Dum-dum fever and kala-azar, and as soon as opportunity offered, set off to Assam, where he made spleen-smears from a number of cases of kala-azar and in all of them found Leishman-Donovan bodies. His carefully prepared paper was sent to the Indian Medical Gazette[9], but alas, he was forestalled. Ross had been in touch with a colleague then in India, C. Bentley, and had asked him to examine spleen smears from cases of kala-azar occurring in an area where this disease was endemic. The findings were of course positive, and Bentley cabled this fact to Ross, who immediately wrote to the British Medical Journal[10] announcing this discovery. This letter was published in January 1904, some months before Rogers' much more detailed account appeared.

It was left to Rogers[11], however, to produce evidence which left little doubt that Leishman was right in suggesting that the parasites he had discovered were related to trypanosomes. Sensing the importance of Leishman's suggestion that there might be some relationship between cercomonads and Leishmania parasites, Rogers attempted to culture the parasites recovered by spleen puncture from cases of kala-azar. His first experiments, carried out at a temperature of 37°, were unsuccessful. Arguing that the disease was almost certainly transmitted by an insect, and that some development of the parasite might well occur in this cold-blooded vector, he kept his cultures at room temperature, and was immediately rewarded with success. The small oval bodies elongated and developed a terminal flagellum, and in this flagellate form underwent rapid division. These flagellate parasites, now known as leptomonads, were clearly close relations of the trypanosomes, and had nothing in common either with *Sporozoa* or *Piroplasmata*.

In July 1904, at the Annual Meeting of the British Medical Association, the Tropical Diseases Section devoted its sessions to a symposium on Leishmaniasis, and rarely can there have been such a gathering of tropical talent. The opening paper by Leishman himself was an elegant summary of the known facts. He was followed by Rogers, who gave the clinical picture of kala-azar and the results of his culture experiments, which apparently were regarded with some scepticism, for Rogers was then relatively unknown. Donovan, who followed, accepted Laveran's *Piroplasma* hypothesis. Bentley still advocated Ross's conclusion that the parasite was a sporozoan. Christophers, Castellani, and Manson all took part in the discussion and the last word lay with Sir David Bruce, who strongly supported Leishman in every way, and indeed held that all the credit for this discovery should go to Leishman, and that ' donovani ' should be omitted from the name.

The admission of another case of Dum-dum fever to Netley Hospital in 1904 gave Leishman an opportunity to add to his personal observations. A study of the clinical features of the case enabled him to state that " the conclusion can hardly be avoided that Dum-dum fever may eventually prove to be no more and no less than kala-azar ", though he justified the name of Dum-dum fever as a temporary label. He advocated spleen puncture to confirm the diagnosis, but apparently the spleen " was not accessible ". Ultimately the patient died, and spleen smears taken at autopsy showed numerous leishmania parasites, while cultures confirmed Rogers' earlier findings, and definitely established Leishman's conclusion that the parasite belonged to the trypanosome family. He noted that the Leishman-Donovan bodies were widespread throughout the body, but in sections (as opposed to smears) were always intracellular. For various reasons he concluded, and concluded rightly, that the parasites of kala-azar and tropical sore, though similar in many ways, were not identical.

Though Leishman had no further opportunity for original work on the parasites of kala-azar and tropical sore, he maintained a close interest in the subject and in 1911-12 published, in the Journal of the Royal Army Medical Corps, and in the Quarterly Journal of Medicine, comprehensive and up-to-date reviews. By this time the flea had been incriminated as the dog-to-dog vector of Mediterranean kala-azar, and the sandfly had come under suspicion as the vector of tropical sore. Many years were to pass before Sinton, Napier and others by circumstantial evidence, pinpointed *Phlebotomus argentipes* as the carrier of Indian kala-azar, and not until 1942, when Shortt and his colleagues[13] discovered the feeding technique which allowed the parasite to develop to the infective stage, was experimental infection of man by the bite of the sandfly successfully accomplished. Today research is focussed on the discovery of reservoirs of infection in small mammals, and in the identification of the particular sandfly species of which act as vectors in the different countries in which leishmania infection occurs. The story begun by Leishman over 60 years ago has not yet reached its final chapter.

Spirochaeta Duttoni

Leishman was greatly interested in spirochaetes of all kinds, and at one time or another worked with those causing syphilis, yaws, Vincent's angina, phagedaenic tropical ulcer, and in particular with *Spirochaeta duttoni*, as it was then called, which is the infective agent of Central African relapsing fever. The names of these parasites have been changed since Leishman's time, but for convenience I propose to use the popular name "spirochaete".

In 1905 Dutton and Todd[14] incriminated the tick *Ornithodorus moubata* as a vector of relapsing fever but rather unexpectedly found that the infection did not seem to be transmitted in the saliva of the tick when it fed on its host. The infective agent, which they could not detect microscopically, was however present in the faeces and coxal fluid excreted by the tick at the end of a blood-meal, and was able to gain access to the vertebrate host's body either through the puncture hole left by the tick's proboscis, or through scratch abrasions. They also discovered the hereditary transmission of the parasite through the eggs of the tick to the larvae and nymphs of the next generation. Koch in 1906[15] found spirochaetes in the body cavity of infected ticks, and also in the ovarian tissue and eggs. Leishman determined to study this subject more thoroughly, and shortly after moving into his new quarters in this building embarked on a series of experiments which continued throughout his active research career. With a batch of ticks sent by a colleague from Nyasaland he succeeded in infecting a monkey, and thereafter maintained this strain of spirochaetes by passage through successive animals. In early experiments, aimed at propagating the spirochaetes in artificial culture, he was unable to repeat Noguchi's reported success, and at best could only keep them active for some weeks, without multiplication, in a rather bizarre medium consisting of broth plus extract of medicinal leeches. He was also unable to confirm Koch's findings, although on one out of many trials he did find spirochaetes in three eggs. He embarked on his major experiments by feeding numerous ticks on heavily infected mice. These ticks were then divided into batches, and exposed to different environmental conditions. Thereafter one tick per day from each batch was carefully dissected and every organ examined in detail, a most laborious and time-consuming task. In this way it was possible to follow the day to day changes in the spirochaetes, though of course the method did not reveal the consecutive stages of development as they occurred in any one tick. In earlier investigations fresh specimens of tick's tissues were examined by transmitted light and smears were fixed and stained by his own stain. Latterly Leishman used dark-ground illumination, and found that this gave excellent resolution even when examining stained specimens which had been prepared years before. His findings can be summarised as follows. The spirochaetes in the tick's intestinal tract gradually lost their motility, and many underwent structural changes such as the formation of granules and the extrusion of buds. They did not multiply in the tick in this form and rapidly disappeared, few being left by the 8th or 10th day. The granules were liberated either by extrusion or by breaking down of the spirochaetes, either in the gut or in the tissues where they had wandered. They occurred as small clumps in a homogeneous matrix, and were found chiefly in the cells lining the Malpighian tubules or in the genital tissues. They persisted throughout the life of the tick, and were to be found at times in the intraovarian eggs as well as in the young nymphs hatched from these eggs. In the young nymphs they were capable of multiplying, sometimes to an enormous extent.

Under certain conditions such as a high temperature, spirochaetes tended suddenly to reappear about 10 days from the date of the infected blood meal. These differed from the blood forms, being smaller, thinner, more irregular in their curves, and more faintly staining. They were actively motile. They arose from granules, and once present persisted throughout the life of the tick. Leishman believed that these " young " spirochaetes, or the granules immediately preceding them, were the infective form.

As regards the actual transmission of infection from tick to mammalian host, Leishman allowed an infected tick to bite a monkey, but removed it after a short interval, no infection resulted. On the other hand, coxal fluid, in which neither spirochaetes nor granules could be identified, proved infective when injected into a mouse. He did not, however, rule out the possibility that infection might be transmitted in the saliva of the tick.

Leishman's work on this subject was interrupted by the advent of the First World War, and was never finished to his satisfaction. In recording his findings in the Horace Dobell lecture in 1920, he stated regretfully that he was unable to present definite conclusions, and considered that more research was necessary before this could be done.

It is remarkable that, more than half a century after Leishman propounded the 'granule' theory, the subject is still controversial. It has been conclusively proved by Adler[16] that the Palestinian tick, *Ornithodorus papillipes* can inject spirochaetes in its saliva, but as already stated, Leishman did not deny that this might occur. Continental workers[17] have recorded their inability to find granules or waves of young spirochaetes as described by Leishman. This, in the face of Leishman's repeated experiments, does not carry conviction. On the other hand, much evidence has been produced, which it would be tedious to recapitulate, but which favours Leishman's theory that the granules represent a stage in the life-cycle of the spirochaete. There has certainly been no positive evidence to disprove it. It is possible that before long the problem may be solved by modern techniques such as fluorescent microscopy, or by the use of radio-active isotopes (though it seems doubtful if an organism which does not multiply in artificial culture can be labelled in this way) or by the electron microscope, which may reveal hidden details. Should investigations along these lines be carried out, there is good reason to hope that they will show that, once again, Leishman was right.

Minor Projects

In addition to these major subjects of research, Leishman from time to time turned his attention elsewhere. He observed some unusual inclusions in the large lymphocytes of a patient suffering from blackwater fever, and thought that they might be parasites of sorts, but further investigations showed this to be a false trail. During the war he co-operated in work on the treatment of war wounds, and assisted Sir David Bruce in his statistical investigation into the efficacy of prophylactic tetanus antitoxin. He also attempted to assess the value of influenza vaccine—a bacterial and not of course a viral vaccine—as used in the 1918-19 epidemic.

The Character of Leishman's Work

The outstanding feature of all Leishman's research work was its thoroughness, which stemmed from the great care he devoted to advance planning and to the tenacity with which he pursued his objective. He was highly critical of his findings, and where the issue was obscure, had no hesitation in repeating the most tedious of his experiments to confirm an unusual or unexpected observation. He had an open mind, and gave careful consideration to criticism from others, but none the less had the courage of his convictions, and when convinced did not deviate from the conclusion he had reached. He did not come quickly to a decision, or rush hastily into print, as witness the delay in publishing an account of the parasites he had found in the spleen smear from the case of Dum-dum fever; but it is noteworthy that the conclusion he reached in this case withstood the criticism of the most eminent authorities of the time. He possessed a high degree of manipulative skill, which he used to good purpose. Few people could have carried out, as he did, the intricate and laborious dissection of several small, tough-skinned ticks per day, repeated day after day, and the very difficulty of the task may in part explain why his results have still to be confirmed. One of his greatest gifts was his command of language, and his power of expressing ideas and observations lucidly, yet briefly. His scientific papers, and particularly his reviews, such as the Harben Lectures and the Horace Dobell Lecture, are remarkable for the ease with which they can be read and understood. They might well be made compulsory reading for the young scientists of the present day.

Leishman as an Administrator

Of Leishman's later work as an administrator little has been written, but the fruits of his labour remain for all to see. He it was who created the Directorate of Pathology, with graded appointments for specialists in this subject and terms of reference which conferred on them a considerable degree of independent responsibility. There were at first grumbles that the pathologist should be in most ways outside the jurisdiction of the Commanding Officer of the adjoining hospital. This, however, was one of the main objects of the scheme, as the old style of Commanding Officer was apt not to be laboratory-minded, and to regard the pathologist as a useful " extra hand " for odd jobs, to the detriment of his specialist work. However, these difficulties soon smoothed out, as the atmosphere changed and the value of the help which the laboratory could give to the clinician became apparent even to the sceptics. But Leishman's interests, particularly when he became Director General, were in no way confined to pathology. Much though we may regret that his productive life as a research worker was so short, for he spent little more than 15 years at the laboratory bench, his later years were no less productive in other ways—ways which greatly enhanced the standing of the Corps as a whole. In the estimation of his civilian colleagues he ranked as a scientist of the first order, and this in itself was of great prestige value. His keen interest in the professional side of Corps activities spread down through all ranks. The teaching of junior and senior officers in the courses of instruction given at the College, remodelled when the institution resumed its normal function after the First World War, reached a standard that had never before been attained. Subjects of military interest were taught by experienced serving officers, while clinical instruction in the more homely conditions common to civilian and soldier alike was given by teachers of the adjoining medical schools. 50% of the officers of each senior course were selected for a period of specialist training in the subject of their choice, either in the College itself, as in the case of Hygiene and Pathology, or under civilian specialists. An equal stimulus was given to the training of other ranks in all the various types of duty they might be called on to perform, such as nursing orderly, theatre orderly, laboratory technician, and so forth. The Corps, in fact, became as never before a specialised professional body to which adverse criticism, common in past years, could no longer be applied. Although Leishman was not the originator of all these schemes, his powerful support did much to hasten their maturation. His premature death in 1926, after serving but three years as Director General, was a tragedy.

Leishman as a Friend

It was my good fortune when a junior officer to have personal contact with Sir William Leishman during the last years of his life. As Director General, he was *ex officio* Chairman of the United Services Section of the Royal Society of Medicine, during which time I was Secretary for the Army. I had therefore, from time to time, to consult him on the business of the Section, and arising out of this, and partly perhaps because we had certain background items in common, he continued to take an interest in my career. My outstanding recollection is of his friendliness and kindness. He was invariably courteous to everyone with whom he came in contact, imperturbable in any of the minor crises which arose, and always completely in command of the situation. No one, indeed, could have been more unlike the irascible senior officer of the cartoonist. Yet his quiet manner stemmed, not from weakness, but from strength; it was the outward sign of the inward poise of a really great man who knows that he has given of his best. The world of medicine as a whole and particularly we as a Corps owe him a vast debt. It is with this very much in mind that we pay homage to him tonight.

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*A bibliography compiled by M. Davies of the works of Sir William Leishman will be found in the Journal of the Royal Army Medical Corps (1961) Volume 107, 89