

## Obituary

### MURIEL ROBERTSON, 1883-1973

Muriel Robertson, protozoologist and bacteriologist, died on 14 June 1973 at the age of 90. Born in Glasgow on 8 April 1883, she was the seventh child of the engineer Robert Andrew Robertson and his wife Elizabeth, who eventually brought up 12 children. The family had connexions in Northern Ireland, and it was a family estate in Limavady for which Muriel had a lifelong affection and at which she spent her last years.

The atmosphere of the Glasgow home was cultivated and stimulating, and it was there that Muriel received a liberal education in literature, the arts, music and languages. She became fluent in French and German, and later in Italian. At this time she had no particular feeling for science, but she absorbed a good deal from her father and his visitors by what she called 'osmosis'. Her first formal experience of scientific matters came when she was working for an arts degree at Glasgow University. Zoology and botany were part of the curriculum, and it was Graham Kerr, her professor of Zoology, who started her, while still a student, on her first research; the life-cycle of *Pseudospora volvocis*, a protozoal parasite of colonies of *Volvox*. After taking her M.A. in 1905, she worked for 2 years in Glasgow and in 1907, on the award of a Carnegie Fellowship, went to Ceylon, where she studied the blood parasites of reptiles - particularly trypanosomes - under Dr Willey, the Curator of the Museum at Colombo. She returned in 1908, and in 1909 joined E. A. Minchin, then professor of Parasitology at the Lister Institute in London, where in 1910 she became a member of the staff.

Muriel Robertson's chief interests, the life-cycles of trypanosomes in reptiles and fish, were to prove decisive in her next move, for in 1911 she was appointed Protozoologist to the Protectorate of Uganda, a position she held for 3 years. At the start of the century there had been a severe outbreak of sleeping sickness in the Protectorate, which had prompted the subsequent dispatch from Britain of three Commissions to study the disease. Muriel joined Dr Lyndhurst Dyke at the Royal Society's laboratory at Mpumu and in the course of her work added Luganda to the languages in which she was fluent. With characteristic intrepidity, she travelled alone through the Ugandan forests, following her native porters on a bicycle, armed with a Männlicher rifle. The crocodile whose skin adorned her laboratory in later years was one which was holding up her porters at a ford.

Muriel Robertson returned to the Lister Institute just before the 1914-18 war and, except for a break during the 1939-45 war, remained there until 1961, some 12 years after her official retirement at the age of 65. Protozoology was her major field; the two wars were the occasion of her two excursions into bacteriology, both of them bearing on the infection of war wounds by clostridia. The break during the second war was spent in work on gas-gangrene at the Institute of Animal Pathology in Cambridge. Towards the end of her time at the Lister Institute, she suffered from acute glaucoma and lost one eye as a consequence. With characteristic courage and tenacity she continued to work, removing in 1961 to Cambridge where, at the A.R.C. Institute of Animal Physiology, she tried to complete her studies on *Trichomonas*; but after 18 months she could no longer work to the high standard she demanded of herself. Moreover, the physical condition which had precipitated the

The Editors regret the delay in publishing this obituary notice.



DR MURIEL ROBERTSON

glaucoma began to tell on her, and she retired to the family home at Limavady, to live with her sister Dorothy. For many years she kept in touch with her family and friends by annual visits to England. These ceased, and in a few years she died after an 18 months' illness in a hospital at Limavady.

#### *Work on protozoa*

When Muriel Robertson started her first major parasitological study, on the development of a parasite of the skate, *Trypanosoma rajae*, in the leech *Pontobdella muricata*, the complex cycle which the trypanosomes undergo in the vector before becoming infective for the alternative host had not been discovered. Her establishment of the cycle with *T. rajae*, and that of *T. vittatae*, a leech-borne parasite of the aquatic tortoise she worked with in Ceylon, illustrates the qualities that made her a great protozoologist – brilliant microscopy, the capacity for long hours of acute observation, and a flair for the interpretation of morphological changes. She had no facilities for clinching her observation on the two cycles by experimental transmission to uninfected hosts, but in a study of a goldfish trypanosome, she obtained populations of uninfected goldfish and of the leech vector, with which she proved transmission, not only of the goldfish trypanosome, but of related trypanosomes from bream and perch, to the goldfish.

The culmination of these studies was in her outstanding work on *T. gambiense* in Uganda, described in five papers of which the last, published in 1913, contains the quintessence of her work on the life-cycle of the trypanosome in the blood and the tse-tse fly. It is a classic; not only for the elegance of her procedures and of the illustrations, but for the soundness of her

observations. Many of them were later to be confirmed by those with modern cytochemistry and electron microscopy at their disposal. Perhaps the most original feature of this work was her description of the sequence of developmental stages in the fly and the pathway by which ingested trypanosomes finally reached the salivary glands, in a form infective for the mammalian host – a discovery that had hitherto eluded David Bruce and his co-workers in their extensive work on *T. gambiense* in the tse-tse fly in Uganda.

As an experimental vertebrate host, Muriel used a Cercopithecus monkey. There she noted the alternating periods of abundance and scarcity of the parasite in the blood which characterize a number of parasitaemic infections. She failed to discover any extravascular foci of infection which might be the source of renewed parasitaemia after a depressed period, and considered that the diminutions of parasitaemia were due, not to phagocytosis, but to destruction of the trypanosomes by the blood plasma. Her suggestion that changing degrees of parasitaemia reflected changes in the balance between the host's capacity to destroy the trypanosomes – expressible by the blood itself – and the parasite's capacity to maintain itself, anticipated the modern view that they are determined by periodic killing by antibody specific for the parasite's antigens, followed by the emergence of antigenic mutants – which are in turn destroyed when the host's immune response to the new antigens has matured. She also observed that the blood was infective for the tse-tse fly only in the depressed periods of parasitaemia, when of the two main forms of trypanosome – long and slender, and short and stumpy – only the stumpy form persisted; and postulated that only the latter were infective – an hypothesis that was later to be confirmed by others.

The 1914–18 war interrupted Muriel's protozoology and launched her on her first bacteriological phase, which lasted to the mid-1920s. She then applied the recently devised Feulgen stain for DNA to the flagellate *Bodo caudatus* and to *T. rajae*, and made a number of observations on the structure of their kinetoplasts and nucleoli. Her paper, published in 1927, on this subject was important not only for the results, but because it introduced the method to other protozoologists. Her next work was on trypanflavine resistance in *B. caudatus* in which she used the disappearance of the kinetoplast as an index of drug action. By analysis of clones isolated from mass culture she showed that resistance was not a qualitative variate, but a quantitatively variable feature of the individuals in a population. The drug appeared both to select resistant variants and to modify the flagellates growing in the presence of the drug.

Her post-war investigations of the clostridia had introduced her to the serological analysis of microbial antigens, a technique which she then applied to Bodo and a ciliate of the Glaucoma group. Rabbit serum contained low-titre 'normal' agglutinin for Bodo, and anti-Bodo sera, a high-titre agglutinin. Both were lytic in the presence of fresh serum. A similar pattern obtained with Glaucoma. Here she found two antibodies, one to a heat-stable antigen which stimulated the organism to secrete an enveloping sheath from which living ciliates then escaped; and one to a heat-labile antigen, which was the lysin.

The work led to her major achievement in the immunology of protozoa. In 1938 she began a collaboration with W. R. Kerr, of the Veterinary Research Laboratory in Belfast, on the infection of cattle by *Trichomonas foetus*. The combination of Dr Kerr's experimental and veterinary skills and Muriel Robertson's protozoological and serological experience was, over the next 20 years, to elucidate most of the important immunopathological features of the disease. A diagnostic agglutination test with living, artificially grown *T. foetus* was devised (*T. foetus* was at the time thought to be an antigenically homogeneous species; later two serological varieties were distinguished). All cattle sera, and indeed those of horses, sheep, goats and men, were found to contain low-titre agglutinins, lytic in fresh serum.

Their origin was obscure; they appeared in the calf at 5 to 7 weeks, apparently without any exogenous antigenic stimulus, and were passed from mother to newborn calves *via* the colostrum. High-titre agglutinins, likewise lytic, appeared after infection – though not in all infected animals – and, unlike the ‘normal’ antibody, could induce passive anaphylactic hypersensitivity.

Experimental infection proved to be possible only by intra-uterine instillation of *T. foetus* during oestrus. As with the natural disease, it resulted in either abortion or infertility. In unmated animals, an intra-uterine inoculum largely disappeared within 3 weeks, a self-sterilizing action which was exploited for the eradication of the disease. Potentially infected females were held unmated for 6 weeks to free them of infection and the main reservoir of infection, *T. foetus* in the preputial sheath of the bull’s penis, was eliminated by killing all male carriers of the organism. By this means, trichomonad infertility and abortion disappeared from Northern Ireland within 3 years.

The erratic appearance of circulating antibody after natural or experimental uterine infection, in spite of a raised immunity to further intra-uterine infection; the occurrence of allergic hypersensitivity of the genital tract in the absence of circulating antibody; and the failure of abundant circulating antibody induced by intramuscular immunization with *T. foetus* to protect against genital infection; all indicated that locally produced antibody played a part in specific immunity to the trichomonad. This indication was confirmed by demonstrating specific muco-antibody in the uterine and vaginal secretions of immune animals.

The picture of the immune response to uterine infection by *T. foetus* that finally emerged was of a natural infection confined to the genital tract. Some trichomonad antigens might be absorbed, resulting in a systemic induction of agglutinating and hypersensitizing antibody. The hypersensitivity was at least partly anaphylactic in type, since it was diminished by anti-histamine drugs. It also disappeared from immune animals during and immediately after parturition, presumably owing to an outpouring into the blood of corticosteroids, since injected corticosteroids would desensitize non-parturient hypersensitive animals. The circulating antibody did not reach the uterine cavity, where immune and local hypersensitivity were due entirely to a locally produced antibody, demonstrably lethal for *T. foetus*. Here was a clear-cut demonstration by the two collaborators of a local antibody immunity of the kind that had, for some years, been postulated for gut infections by dysentery bacilli and the cholera vibrio. They made another contribution to general immunology: they observed that the post-natal calf made no immune response to doses of intramuscular antigen effective in adult cattle, and no immediate response to larger doses though, as the calf became immunologically mature, some antibody was produced. This suggestion of immunological paralysis was confirmed by a demonstration that in the 3-week-old calf, large doses of antigen impaired the response to *T. foetus* for up to 26 months.

#### *Work with bacteria*

In 1914 and 1939, Muriel Robertson turned from protozoology to work more immediately relevant to war; on both occasions the anaerobic clostridia claimed her attention. In World War I, she moved from studies of infected material from war-wounds to the better identification of what were later to emerge as *Cl. welchii* (perfringens) and *Cl. septicum*; and so made her contribution to the current British work that was to establish these two organisms, and *Cl. oedematiens*, as the causes of gas-gangrene in man. She tried, and failed, to immunize mice with heat-killed *Cl. welchii*. The impression of the muddle in which inadequate techniques of obtaining pure cultures had landed the anaerobic bacteriology of the day never

quite left her, and in later years she would emphasize her disbelief in the purity of other workers cultures by declaring there was no such thing as a culture of *Cl. welchii* unmixed with at least a latent *Cl. sporogenes*. Apart from a demonstration that toxigenic *Cl. tetani* was present in 3.5 % of wounds of soldiers without tetanus, as opposed to 50 % of those with the disease, her remaining work was largely with *Cl. septicum* – its identification in wounds, its mode of infection in mice and its serology. She decisively separated it from *Cl. chauvoei* by agglutination reactions and established four serological types, all producing the same exotoxin. With Arthur Felix, she distinguished flagellar and somatic antigens and, somewhat surprisingly, showed that somatic antibody gave better passive protection than antitoxin against *Cl. septicum* infection. She also wrote the section on the bacteriology of anaerobic infections for the official history of the war (1923), and the chapter on gas-gangrene organisms in the M.R.C. System of Bacteriology (1929).

The period 1939–45 was one of collaboration, in Cambridge, with James Keppie. They investigated the sensitivity of *Cl. welchii* to the newly available sulphonamide drugs, and established that *in vitro* toxin production was no guide to the apparent pathogenicity of a strain in man; highly toxigenic strains, for example, might be mere transient contaminants of a wound. They also undertook to confirm, with material made available by other British workers, the American reports that mice could be immunized with the toxoids of *Cl. welchii*, *Cl. septicum* and *Cl. oedematiens*. The immunity was truly antitoxic, in that the blood of the animals contained no detectable somatic antibodies and, with *Cl. welchii* and *Cl. oedematiens* at least, that protection was roughly correlated with the concentration of circulating antibody.

Muriel Robertson had great natural gifts. She received the upbringing of an Edwardian gentlewoman and a liberal education in the arts, from which she emerged as what is best described as a patrician intellectual. She was impeccably polite and expected good manners in others; but she did not suffer gladly anyone she thought to be a fool or intellectually pretentious. In scientific controversy she was sharp and ruthless – sometimes unfairly – especially when she felt that some work or an argument was slipshod. She could be tart about established scientific reputations, perhaps the result of having had in her early days to assert herself at a time when women were a rarity in a masculine world of research.

Conservative by nature, she regarded pre-1914 Europe as a model of what international science should be and deplored its disappearance. Nevertheless, she came to terms with the changing world and her adaptability is nowhere better illustrated than in her scientific achievement. It is remarkable how, rooted in observational protozoology, she moved so effectively into experimental bacteriology and immunology. If the success of these moves is in part due to her collaborators, she must be credited with the prescience to chose those with the experience that would complement her own. In these, and in many of her pupils and closer colleagues, she inspired admiration and affection – she was particularly at ease with younger colleagues.

Muriel had none of the aggressiveness associated with the traditional blue-stocking. She was thoroughly feminine in appearance and manner, and embroidery and flowers were among her relaxations. Her conversation was rewarding, for she had humour and wit as well as learning; but it could be rather one-sided and sometimes exhausting. Her enthusiasms prompted a rather didactic flow of fact and opinion about art, science, literature, music and history, which advanced almost by a process of free association, new subjects starting up even before the sentence about the current subject was completed. Her didacticism was not

all unconscious; for she felt with younger scientists at least that she should broaden their horizons beyond those acquired in a narrowly specialized education.

Muriel Robertson was elected F.R.S. in 1947. She was a fellow of the Royal Society of Tropical Medicine and the Institute of Biology – and a member of the Pathological Society, the Society for Experimental Biology and the Medical Research Club. She was one of the founders of the Society for General Microbiology and in its early days, from 1945 to 1948, served on its Council. The Society made her an Honorary Member in 1962, and invited her to be the Marjory Stephenson Memorial Lecturer in 1963, when a brief appreciation of her appeared in the *Journal of General Microbiology* on the occasion of her 80th birthday. In her last years at the Lister Institute, when she was the only remaining scientifically active representative of the great ladies of the Institute's pioneering days under Charles Martin, Muriel herself became something of an institution, with very definite views about things and persons. Her disapprovals were perhaps more mellowly expressed, but nonetheless decided. With the many, both in the Institute and outside it, whom she accepted as part of her world she was, and remained, a charming companion and friend.

I am indebted to Dr Ann Bishop for material about M.R.'s trypanosomiasis work, of which a fuller critical account appears in the Memoir written by us in *Biographical Memoirs of Fellows of the Royal Society* (1974) **20**, 317–347. A. A. MILES

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*Bacteriology*

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