ELSEVIER

Toxicon 48 (2006) 738–754

TOXICON

www.elsevier.com/locate/toxicon

# Twentieth century toxinology and antivenom development in Australia

Kenneth D. Winkel[a,*], Peter Mirtschin[b], John Pearn[c]

[a]*Australian Venom Research Unit, Department of Pharmacology, University of Melbourne, Parkville, Vic 3010, Australia*
[b]*Venom Supplies Pty Ltd, PO Box 547, Tanunda, South Australia 5352, Australia*
[c]*Department of Paediatrics and Child Health, Royal Children's Hospital, Brisbane, Qld 4029, Australia*

Available online 9 August 2006

**Abstract**

It was not until the last decade of the 19th century that an experimental approach (led by Bancroft in Queensland and Martin in Sydney and Melbourne) brought a higher plane of scientific objectivity to usher in the modern era of Australian toxinology. This Australia era, 1895–1905, coincided with and in some respects was the result of the new knowledge emerging from Europe and the Americas of the therapeutic effects of antitoxins. The subsequent systematic study of Australian venoms and toxins through to the 1930s and beyond, by Tidswell, Fairley, Ross, Kellaway and Cleland, set the foundation for Australia's leading reputation in venom research. As elsewhere, this development was to revolutionise the medical management of those victims who in the past had died in Australia from our venomous and toxic fauna. Morgan, Graydon, Weiner, Lane and Baxter at the Commonwealth Serum Laboratories emphasised the importance of cooperation between those expert at catching and milking the venomous creatures and those developing the antivenoms. Commercial antivenom manufacture began in Australia in 1930 with the tiger snake antivenom. This was followed by other antivenoms for the other important species (1955: taipan; 1956: brown snake; 1958: death adder; 1959: Papuan black snake; 1961: sea snake; 1962: polyvalent) including the first marine antivenoms in the world (1956: stonefish antivenom; 1970: box jellyfish) culminating, in 1980, with the release of the funnel web spider antivenom. More recent activity has focused on veterinary antivenoms and production of new generation human antivenoms for export (CroFab and ViperaTAB). This paper reviews some of the milestones of Australian toxinology, and antivenom development in particular, during the 20th century.


*Keywords:* Toxinology; Australia; Antivenoms (antivenene); Snakebite; Poisonous plants

## 1. Introduction

Aboriginal men and women have lived with the world's most venomous creatures, on the land and in the seas of Australia, for some 60 millennia prior to European settlement in 1788. Across Australia, Aboriginal men and women of more than 600 language groups had developed an intimate knowledge of the toxic biota, had learned to respect it and mostly to avoid its threat (Pearn, 2001; Pearn and Winkel, 2006). For a century after colonial settlement, European scientists brought the "new" zoological and botanical knowledge to scientific notice in the wider world. Medical practitioners in particular—naval and army surgeons, surgeon-expeditionary and medical

*Corresponding author.
*E-mail address:* kdw@unimelb.edu.au (K.D. Winkel).

0041-0101/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2006.08.001

immigrants—established the discipline of Australian toxinology (Pearn, 1994a, b; Pearn and Winkel, 2006). This "new" knowledge of Australia's toxic biota was essentially observational, descriptive and empirical (von Mueller, 1858–1882; Krefft, 1869; Koch, 1871–1877). It was not until the last decade of the 19th century that an experimental approach (Bancroft, 1894; Lauterer, 1895; Martin, 1897a, b; Tidswell, 1899, 1900) brought a higher plane of scientific objectivity to this subject and so ushered in the modern era of Australian toxinology. This Australia era, 1895–1905, coincided with and in some respects was the result of the new knowledge emerging from Europe and the Americas of the therapeutic effects of passive immunisation with antivenene. As elsewhere, this development was to revolutionise the medical management of those victims who in the past had died in Australia from our venomous fauna. This paper reviews some of the milestones of Australian toxinology, and antivenom development in particular, from that time (Table 1).

## 2. A universal antivenom?

Antivenom therapy was made possible by Roux's and later Calmette's (1894) and Brasil's (1898) (Hawgood, 1992) development of passive immunisation, discoveries made possible in turn by Pasteur's (1881) earlier demonstration of active immunisation and protection of sheep against the Anthrax bacterium. The first specific application of Pasteur's principles to snake antivenom production began with Henry Sewall's experiments in Michigan whereupon he successfully injected increasing amounts of rattlesnake venom into pigeons without ill effect (Sewall, 1887). Within a few years Albert Calmette (1863–1933) had progressed this idea at the Institut Pasteur in Saigon and later in Paris and Lille (1891–1896), leading to the world's first commercially available antivenom (Calmette, 1894). The general principle of Calmette's specific discovery—that the serum of horses immunised against cobra venom would universally protect the snake bitten patient—was quickly tested in Australia.

Table 1
A chronological summary of the development of passive immunotherapy and the introduction of commercial antivenoms for the management of human envenomation in Australia

| | |
|---|---|
| 1930 | Tiger Snake (*Notechis scutatus*) antivenom. |
| 1938 | Tick (*Ixodes holocyclus*) antivenom. |
| 1956 | Red-back Spider (*Latrodectus hasselti*) antivenom. |
| 1955–62 | Species-specific snake antivenoms. |
| 1955: | taipan (*Oxyuranus scutellatus*). |
| 1956: | brown snake (*Pseudonaja textilis*). |
| 1958: | death adder (*Acanthophis antarcticus*). |
| 1959: | black snake (*Pseudechis papuanus*). |
| 1961: | sea snake (*Enhydrina schistosa*). |
| 1959 | Stonefish (*Synanceia*) antivenom. |
| 1962 | Polyvalent Snake antivenom introduced. |
| 1970 | Box jellyfish (*Chironex fleckeri*) antivenom. |
| 1980 | Funnel-web Spider (*Atrax robustus*) antivenom. |

Commercial antivenoms approved for human use in Australia have all been produced by the Commonwealth Serum Laboratories (now CSL Limited), Parkville, Australia (after Sutherland, 1994). The dates provided represent the first recorded sales of the respective antivenoms as documented by Sutherland (1994) or in records held by the Australian Venom Research Unit.

Although McGarvie Smith undertook some work immunising rabbits with tiger snake venom in Sydney in 1892, and Thomas Lane Bancroft did likewise with guinea pigs in Brisbane in 1893 (Bancroft, 1893; Cann, 1986), the first major work on antivenoms in Australia came from CJ Martin. The mid-1890s, the British born Dr. (later Sir) Charles Martin (1866–1955) (Fig. 1), initially working as a Demonstrator in Physiology at the University of Sydney and later as an acting Professor of Physiology at the University of Melbourne, challenged Calmette's concept of the universality of his "antivenene". Martin tested it against the venom of both the Australian red-bellied black snake, *Pseudechis porphyriacus*, and that of the common tiger snake, *Notechis scutatus* (Martin, 1897a). He was unable to demonstrate any clinically significant venom neutralisation by this "antivenene" against these two Australian species, thus "disposing of Calmette's concept that his antivenom could be used globally" (Sutherland, 1994). Despite this, "antivenom serum" from Burroughs Wellcome and Co, London, continued to be advertised for sale in the Australasian Medical Gazette into the 20th century (see April 21, 1902 Edition) and Calmette's serum was reported as being used in Australia in 1902 (Bill, 1902). Martin made numerous other contributions to the nascent field of Australian toxinology (reviewed in Hawgood, 1997) including the first investigations into the chemistry of Australian venoms, studies of the pharmacological action of venom, particularly the effect of snake venom on blood clotting, and the nature of toxin–antitoxin relationships as well as the physiology, particularly heat regulation, of marsupials and monotremes, such as the platypus (Martin, 1892;

Fig. 1. Charles James Martin (1866–1955), FRS, pioneer Australian toxinologist and immunologist, former director of the Lister Institute, "a model of what a medical academic should be" (MacIntyre and Selleck, 2003). *Source*: Photograph of portrait donated to the Royal Society by the Lister Institute in 1980. Portrait by M Lewis and reproduced with permission of the Royal Society (© The Royal Society).

Martin and Smith, 1892; Martin and Hill, 1894; Martin, 1895, 1897b).

These studies, aided by his acute observations of slight (but clinically insignificant) protective properties of Calmette's serum against some components of Australian snake venoms, led to both practical and theoretical advances. In practical terms Martin recommended that "curative serum" should be administered by intravenous, rather than subcutaneous, injection (Martin, 1896). He inferred that the antitoxin must be a large molecule that slowly diffused in tissues (Martin, 1897b) and that the volume of antivenom must vary commensurate with the dose of venom injected (Martin, 1898). Martin contributed to a wider understanding of the toxin–antitoxin reaction by confirming the theory of Behring, against the ideas of Calmette, Roux and Metschnikoff, concerning the nature of antitoxins. Behring held that this interaction was a direct chemical one, whereas Calmette thought that the antivenom acted indirectly, enhancing the resistance of the body to the action of toxins (Martin, 1898). It is notable that Martin achieved all this "ingenious and influential research' in Melbourne despite "meagre facilities" and the fact that his infamous predecessor, Halford, drew his annual pension from the funds available for Martin's salary (MacIntyre and Selleck, 2003). He became, in the eyes of the students, "a model of what a medical academic should be" (MacIntyre and Selleck, 2003).

By 1901 Martin was appointed a full Professor at the University of Melbourne and elected Fellow of the Royal Society. His citation for the latter stated "his original papers deal with the chemistry and physiology action of snake venom, and with the action and reaction of toxins and antitoxins". In 1903, Martin resigned to become the Director of the Jenner (subsequently renamed Lister) Institute of Preventive Medicine in London. In 1951, the National Health and Medical Research Council created the CJ Martin Fellowships to recognise how Martin's contribution "laid a solid foundation to research in this country" and how Australian researchers remember him as "one of their most distinguished Masters" (Chick, 1956) who "left behind him a legend of wisdom, integrity and good fellowship that no other "medical academic" ever rivalled" (Burnet, 1971a). He was also a mentor to many of our most eminent toxinologists, particularly Fairley and Kellaway who both worked under his supervision at various times and who were both to benefit from his sponsorship.

## 3. Proof of principle

In 1898, Dr. Frank Tidswell, Principal Assistant Medical Officer of the New South Wales (NSW) Government, undertook pioneering experiments of active immunisation using tiger snake venom. It was through this portal of venom toxinology that Tidswell and Martin became the two pioneer immunologists in Australia. In 1899, Tidswell published the first of two reports, "Report on the Protective Inoculation against Tick Fever: an account of an experimental inquiry into its effect on cattle, and on meat and milk" (Tidswell, 1899, 1900). More importantly for toxinology, in 1901, Tidswell produced Australia's first experimental antivenom by immunising a horse with increasing doses of *N. scutatus* venom. Over a period of three and half years a total of 10 g of venom was injected into this horse. Like Martin with Calmette's

antisera, Tidswell found this antiserum ineffective, in animals, against other venoms such as those of the brown and black snakes (Tidswell, 1902, 1906). Nevertheless he was cautious about the immediate clinical relevance of these results derived from experiments in rabbits (Tidswell, 1902). Unfortunately for the snake bitten of Australia, Tidswell was never to expand the production of this product but was diverted to microbiological projects favoured by the NSW Department of Health. Tidswell also worked indefatigably on the biology and toxinology of a variety of Australian elapids, on the venom of the "Red Spotted Spider" (*Latrodectus hasselti*) and on that of the male platypus (Tidswell, 1906). The latter work, in collaboration with CJ Martin, was actually first reported to the Linnean Society of NSW in 1895. Tidswell also published the first data on the venom yields of Australian snakes (Tidswell, 1902) and developed the first systematic database concerned with the epidemiology of snake bite in Australia. This included "a scheduled set of questions drawn up for the guidance of the police in making their inquiries; previous reports being brought into line by transcription to similar schedules" (Tidswell, 1906). These general principles continue to be applied in today's prospective studies in toxinology.

From the outset, the development of immunotherapy and the specific production of antivenoms proved to be an expensive process. Antivenom research requires both large resources and the potential for co-ordinated inter-disciplinary collaboration between scientists, clinicians and herpetologists. In international terms, the Pasteur Institutes at Paris, Saigon and Lille had demonstrated what could be achieved by such collaboration, if adequately funded and resourced. The Haffkine Institute, the Brazilian Institute Butantan, the Red Cross in Bangkok and four Australian institutes became involved in the production of antivenoms that were to save countless lives.

In Australia, Martin and Tidswell's pioneering work was followed by Dr. (later Sir) Ian Clunies Ross (1899–1959) working on Australian paralysis tick, *Ixodes holocyclus*, antivenom research and development at the Council for Scientific and Industrial Research (CSIR, the forerunner to the current CSIRO) from 1926. Most significantly of all, research led initially by Frank Morgan, at the Commonwealth Serum Laboratories (henceforth abbreviated to "CSL") in Melbourne was to provide safe and efficient antivenoms for widespread use in the Pacific region. Work by Charles Halliley Kellaway (1889–1952) (Fig. 2) and Neil Hamilton Fairley (1891–1966) (Fig. 3) at the Walter and Eliza Hall Institute in Melbourne complemented the antivenom work at these other institutions. This combined output contributed to the fundamental biology of venomous creatures, to clinical research of their envenomation syndromes and to the development of antivenoms to treat them.

Fig. 2. Charles Halliley Kellaway (1889–1952), FRS, military cross winner, third director of the Walter and Elisa Hall Institute of Medical Research, a man of "untiring devotion to duty" who laid the foundation for our understanding of most Australian animal toxins. *Source*: Photograph by Walter Stoneman, Godfrey Argent Studio, London, date unknown (probably late 1940s). Reproduced with permission of the Royal Society (© The Royal Society).

## 4. Modern Australian antivenom development

Central to the development of safe antivenoms has been the work of CSL. This organisation was established in 1916 "to ensure the supply of essential biological products for national health needs, to conduct research and development relating to

Fig. 3. Neil Hamilton Fairley (1891–1966), FRS, "the success which he achieved is woven into the modern practice of tropical medicine" (Boyd, 1966), stimulated the commercial development of antivenoms in Australia. *Source*: Photograph by Walter Stoneman, Godfrey Argent Studio, London, 1946. Reproduced with permission of the Royal Society (© The Royal Society).

biological products and allied fields and to maintain potential production capacity for use in emergencies". As the appointment of a new director of the Hall Institute was in abeyance until after the First World War, the initial appointee being killed at Gallipoli, space in the new institute became available, rent-free, for the use of CSL to produce "biologicals" such as diphtheria antitoxin. So began the nexus between these two great institutions. The pioneers of CSL's antivenoms included Frank Morgan, John Graydon, Saul Wiener, Mervyn Hinton, Bill Lane, Harold Baxter, Struan Sutherland, Alan Coulter and Rodney Harris. A particular feature of the Australian antivenoms has been their evolving biological purity, particularly in terms of the progressively reduced risk of acute intra-transfusion side effects and of anaphylaxis. Currently, the risk of anaphylaxis or anaphylactoid reactions following the use of CSL monovalent antivenoms is reported as less than 10% (CSL Limited, 2001).

In the 1920s, CSL did not have a research role in Australia. Indeed it is hard to appreciate now but at that time medical research in general in Australia was essentially a part-time activity. It was Charles Kellaway at the Hall Institute who "set in motion a tradition of full time research in the biomedical sciences" and "set Australia on a new path to achievement in medicine" (Burnet, 1971c). Melbourne-born, he graduated in medicine from the University of Melbourne in 1911 and went on to apply for a commission in the Australian Imperial Force in 1915 and become a veteran of France (as a Regimental Medical Officer) where he earned the Military Cross (Dale, 1953). He was discharged from active service after being gassed in France and eventually became involved in research on the physiology of high altitude flying with Sir Henry Dale, Britain's leading pharmacologist. After a brief respite in Australia in mid-1919 as a Professor of Physiology at the University of Adelaide, Kellaway returned to the UK to continue his research career, especially at the National Institute for Medical Research in London (Burnet, 1971c, Dale, 1953). As a Foulerton Student of the Royal Society, 1920–23, stimulated by Dale, Kellaway published important papers on anaphylaxis and the adrenal glands (Kellaway and Dale, 1921). He was invited back to Melbourne in 1923 to head the Hall Institute after the previous Director's resignation. Kellaway formed three departments at the institute, then housed within the clinical pathology department in the old Melbourne Hospital: physiology and pharmacology, with himself as head, bacteriology, headed by Macfarlane Burnet and biochemistry, headed by Henry Holden, formerly of Cambridge. As the third Director of the Hall Institute, Kellaway came to his definitive scientific work with the brief return of Neil Hamilton Fairley to Melbourne in 1927.

## 5. The "First" antivenom—the Kellaway years

Fairley brought with him an interest in hydatid disease and snakebite and the latter interested the Commonwealth Government sufficiently for it to award the Hall Institute their first research Grant (£2500) from the Department of Health (Burnet,

1971c). Dr Frank Morgan, newly appointed Director of CSL in 1927, had submitted to the Director General of Health an outline for research work, which suggested, among other things, research into the chemical nature of venoms from Australian snakes, production of antiserum and the practicality of preparation of a polyvalent antivenom (Brogan, 1990). So work began in 1928 with collaboration between Fairley, Kellaway and Morgan. Fairley, "always a man to get things done without delay" won the cooperation of Melbourne Zoo "snake-house" and their snake catcher, Tom Eades (Burnet, 1971d). Within eight months they had milked over 300 snakes and, by October 1930, the first tiger snake antivenom was produced. By 1932, the manufacture of this antivenom was routine and experimental work had commenced on death adder and copperhead antivenoms (Brogan, 1990).

Whilst the first "official" use of an Australian antivenom (that against the tiger snake) was in March 1931 at the Royal Melbourne Hospital (Tisdall and Sewell, 1931), it may well have been used earlier as it was first issued in October 1930. It was certainly used on Kellaway himself at some point in the early 1930s or late 1920s (Burnet, 1971c; Wood, 1984); "Kellaway's researches on the Australian snakes brought problems…Kellaway was bitten by a tiger snake. Fortunately, the first batches of antivenine, made from horse serum, had just arrived. He was injected by Eric Cooper, medical superintendent of the (Royal Melbourne) hospital, and suffered only mild symptoms; all were delighted!". However, Kellaway subsequently "swore that the discomfort of the serum sickness which followed a large intravenous injection of crude antiserum was worse than anything the snake venom could have done" (Burnet, 1971c). Kellaway was known for his swearing, described by Dale as "not always genteel Australian slang" (Dale, 1953).

Unlike Kellaway, Hamilton Fairley, although a critical spur to the development of Australian antivenoms, stayed only briefly at the Hall Institute. A Melbourne medical graduate, Fairley's initial interest in tropical medicine began in Egypt in 1916 as a pathologist, and then physician, in the Australian Army Medical Service and was cemented by association with CJ Martin at the Lister Institute in London in 1919 (Boyd, 1966). Shortly thereafter Fairley returned to Australia and was initially appointed in 1919, as 'first assistant' to SW Patterson, the second Director of the Hall Institute. This appointment, focused on getting complement fixation reactions for syphilis and hydatid disease diagnosis available in Melbourne, only lasted until late 1920 (Burnet, 1971b). Thereafter Fairley left for India where he had been appointed as the Tata Professor of Clinical Tropical Medicine in Bombay. Much to Fairley's annoyance, this position was subsequently withdrawn but he was eventually secured a 5-year position as a Medical Research Officer and consulting physician in Bombay (Boyd, 1966). During these highly productive years in India Fairley developed an extensive interest in herpetology. As Brigadier Neil Fairley, he is best remembered for leading the Medical Research Unit

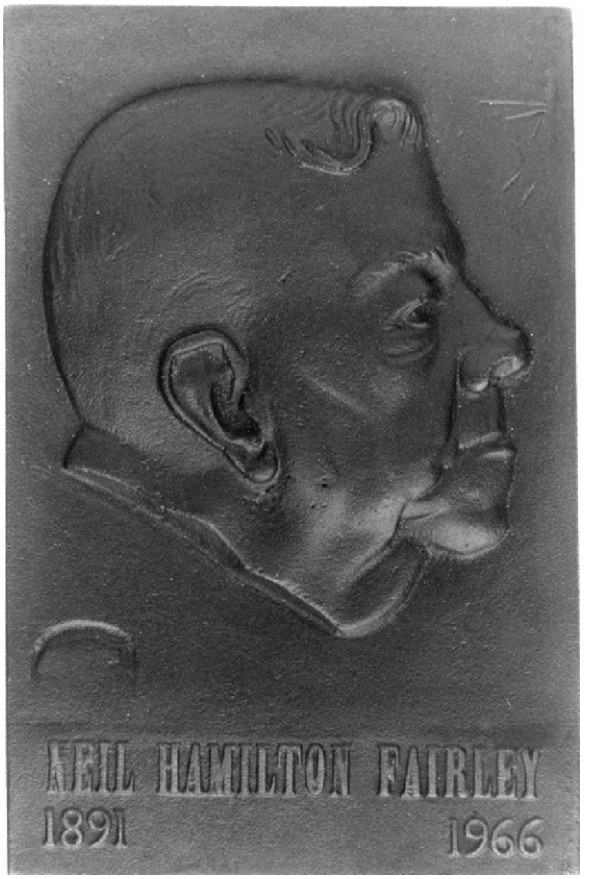


Fig. 4. The Neil Hamilton Fairley Medal, which commemorates the life and works of Brigadier Sir Neil Hamilton Fairley (1891–1966), a pioneer toxinologist, doctor–soldier, physician and leader in tropical medicine. His leadership of research teams during the Second World War was a significant determinant in the Allied victory in the Pacific. This medal, first struck in 1968, is awarded in alternate years by the Royal College of Physicians and the Royal Australasian College of Physicians "to any citizen of any country of any age who has made outstanding contributions to medicine".

attached to Land Warfare Headquarters based in Cairns (1943–46). There, his work on malaria control was one of the major determinants of Allied victory in the Pacific in World War II (Pearn, 2004) (Fig. 4).

During his second 2 years in Melbourne, prior to his move to the Hospital for Tropical Diseases in London, Fairley contributed four major papers on venom research (Fairley, 1929a–c; Fairley and Splatt, 1929). These dealt with the clinical and experimental effects of snake venoms, treatment considerations, including the role of excision and ligature, snake venom yields, and the biting mechanism and dentition of the common venomous snakes. He also recognised the need to define a minimum lethal dose of the venom used in experimental research. His systematic study, much of it of continuing relevance today, led him to conclude that the dangers of any given species varied with "(i) the habits and aggressiveness of the snake (ii) the toxicity of the venom, (iii) the amount available for injection, and (iv) the efficiency of the biting mechanism" (Burnet, 1971d). Of critical importance was his conclusion, from studies of the efficacy of ligatures in sheep and goats, that even the early application of such interventions delayed, but did not alter, the fatal outcome, providing "a clear cut case for mass production of antiserum" (Boyd, 1966). Such was the significance of this work that it was explicitly noted in his citation for his subsequent election to the Royal Society.

By contrast Kellaway continued to work on the pharmacology of venoms until the late 1930s (summarised in Kellaway, 1937a–c; Kellaway, 1939 with a full review in Dale, 1953). Many of his more than fifty papers remain citation classics in Australian toxinology, often continuing as the definitive studies of the pharmacological and physiological effects of Australian snake venoms. He also worked on staphylococcal and mussel toxins, cobra, redback, funnel web, bee and platypus venoms as well as the pharmacological basis of anaphylaxis. Kellaway was also concerned with improving snakebite first aid methods. Important collaborators on Kellaway's work included H.F. Holden (biochemistry), E.R. Trethewie (physiology) and W. Feldberg (notably concerning the "slow reacting substance" of anaphylaxis; Feldberg and Kellaway, 1937, 1938). The latter eventually led Samuelsson and others to the 1982 Nobel Prize for work on leukotrienes and prostaglandins.

Kellaway's distinction in toxinology were such that his 1940 citation for Fellowship of the Royal Society noted that his contributions "on the physiological actions and immunology of snake venoms has made him pre-eminent amongst investigators of this subject". Interestingly, he only ever published two papers with Macfarlane Burnet on toxins but, typical of the latter's interest, it related to the bacterial (Staphlococcal) variety, this form of poisoning being implicated in the Bundaberg disaster (Kellaway and Burnet, 1930; Kellaway et al., 1930). In 1943, Kellaway was invited to become the Scientific Director of the Wellcome Foundation in London and, in accepting the post, recommended Macfarlane Burnet as his successor at the Hall Institute. With this transition, the leadership baton in Australian toxinology moved from the Hall Institute to CSL.

## 6. CSL takes the lead

In 1934, a separate antivenom research department started at CSL, in collaboration with the Hall Institute, with Tom Eades in charge (see Mirtschin, 2006). The main researchers were Jack Graydon and Roland Newton. Unthinkable today, Morgan, the CSL Director, also accompanied Eades on various snake-collecting trips. This collaboration continued until 1941 when routine snake milking at the serum laboratories ceased and by which time Kellaway had ceased venom research and was more focused on military health matters for the war effort (Brogan, 1990; Burnet, 1971c). Interestingly, sufficient para-specificity had been described in the tiger snake antivenom that it was indicated for use in a much broader range of species that it is recommended for today (Kellaway, 1938). The second snake antivenom, against the taipan, was released in July 1955. Major impetus for this development came with the fatal snakebite, in July 1950, to Kevin Budden who provided for the first taipan to be milked for the purpose of antivenom production (see Mirtschin, 2006). Prior to the release of this antivenom, CSL had already developed experimental death adder, black snake, copperhead and brown snake antivenoms but for a variety of reasons, had not produced them commercially. In fact the first horse immunised with death adder venom was bled in December 1929. In 1956, Val Bazley took over from Morgan as Director of CSL and renewed the efforts to finalise the production of a series of important antivenoms Table 1.

Brown snake antivenom was initially hampered by the lack of venom, but this challenge was eventually overcome and the product was released in November 1956 (Sutherland, 1994; Mirtschin, 2006). Similarly, under Bazley the development of death adder antivenom recommenced and it became available by December 1958 (Brogan, 1990). The original black snake antivenom was derived by immunizing horses with Papuan black snake venom (*Pseudechis papuanus*) and was released around the same time as the death adder antivenom (February 1959) (Brogan, 1990). Due to a shortage of that venom, in the late 1970's the immunising venom for the black snake antivenom was changed to that of the mulga snake (*Pseudechis australis*) and has remained so ever since (Sutherland and Tibballs, 2001a). The world's only commercially available sea snake antivenom was first made available for clinical use in December 1961 (provision of the venoms for the former three endeavours is discussed by Mirtschin, 2006; Sutherland, 1994). Prominent additional researchers and managers achieving these milestones were Merv Hinton and Noel Semple (Brogan, 1990; Sutherland, 1994).

The driving motivator and venom supplier for the sea snake antivenom was the late Alistair Reid (1913–83). During his time as consultant physician at Penang General Hospital and as director of the Penang Snake and Venom Research Institute in Malaya, Reid undertook the definitive clinical and epidemiological studies of sea snake envenomation (reviewed extensively in Hawgood, 1998). Reid's Institute provided the beaked sea snake (*Enhydrina schistosa*) venom and ultimately recorded the first instance of the successful use of the CSL sea snake antivenom (Reid, 1962; Kaire, 1964). Baxter, Marr and Lane (then Director of CSL) later demonstrated the very broad specific of the CSL sea snake antivenom (Baxter and Gallichio, 1974), underpinning its place as the only commercial sea snake antivenom in the world (Chetty et al., 2004).

Shortly thereafter (1962) a polyvalent antivenom for Papua New Guinea and Australia was produced (Brogan, 1990). This addressed the challenge of the treatment of the unidentified snakebite, an issue to be later re-examined by Struan Sutherland (see Tibballs, 2006a). Another major contributor to antivenom development at this time was Saul Weiner, who had arrived at the CSL in 1952. Like Sutherland, when he joined CSL he was not primarily engaged in research but he "used his spare time to good purpose" (Brogan, 1990).

Weiner quickly developed the red-back spider antivenom (released and first used in 1956) (Weiner and Drummond, 1956; Weiner, 1956a, b) followed by the world's first marine antivenom—for the stonefish (released 1959) (Weiner, 1959a, c). He also contributed a series of landmark studies concerning these venoms as well as that of the funnel-web spider, and even provided one of the world's first attempts at the active immunisation of humans against snake venom (Weiner, 1959b, 1960, 1961). In something of a parallel to Struan Sutherland's later misadventures, Weiner disagreed with the management style of the CSL director, Val Bazley (Brogan, 1990). He eventually left CSL, in 1962, to train as a clinical allergist and immunologist (Sutherland, 1994) but not before being awarded a Doctor of Medicine by the University of Medicine in 1960, for his thesis on his venom and antivenom studies. Weiner continues in clinical practice and remains actively interested in toxinology and the management of latrodectism in particular (Weiner, 2003).

## 7. Box jellyfish antivenom

The next development was that of the box jellyfish antivenom, work progressed by Harold Baxter, Heather Gallichio and Alex Marr. This product was based on techniques and understanding developed by the pioneer marine toxinologists Southcott, Flecker, Barnes and Endean whose contributions are reviewed in this edition of the journal (Pearn, 2006; Pearn and Fenner, 2006; Hawgood, 2006). In March 1970, CSL reported the first use of this product. In these two North Queensland *Chironex fleckeri* stings the antivenom reportedly had a dramatic effect against the necrotising local tissue reaction (Baxter and Marr, 1970). However, according to unpublished CSL records, this antivenom was first given for systemic illness to a 9-year-old boy at Four Mile beach, Port Douglas, North Queensland on the 29 December 1970 (Winkel et al., 2003). This case was reported to CSL by Dr. Jack Barnes (Winkel et al., 2003). Dr. Barnes noted that, although the culprit jellyfish was not sighted, it was most likely to have been *Carukia barnesi*. Therefore, the two doses of box jellyfish antivenom were administered for what was, in retrospect, a case of Irukandji syndrome. The first intravenous dose was said to have been "ineffective" and, although improvement was seen after the second dose given some 40 min later, Barnes felt

that this was "purely coincidental". The pioneering work of Flecker (Fig. 5), after whom the large multi-tentacled *Chironex* box jellyfish was named, as well as Barnes and Southcott, is discussed elsewhere (Pearn, 1994; Pearn and Fenner, 2006; Tibballs 2006b).

The first case of the use of the box jellyfish antivenom for systemic envenomation after a *Chironex* sting was a 25-year-old man swimming near East Point in Darwin on 29 March 1971 (Winkel et al., 2003). Since these early cases this antivenom has been used in many instance of box jellyfish envenomation (Beadnell et al., 1992; Currie, 1994). The controversy surrounding the place of this antivenom is discussed elsewhere in this edition of the journal (Tibballs, 2006b). Interestingly, Baxter also developed a toxoid, for possible vaccination of high-risk populations. This product was abandoned in the pre-registration testing phase after adverse effects in immunised rabbits (Brogan, 1990).

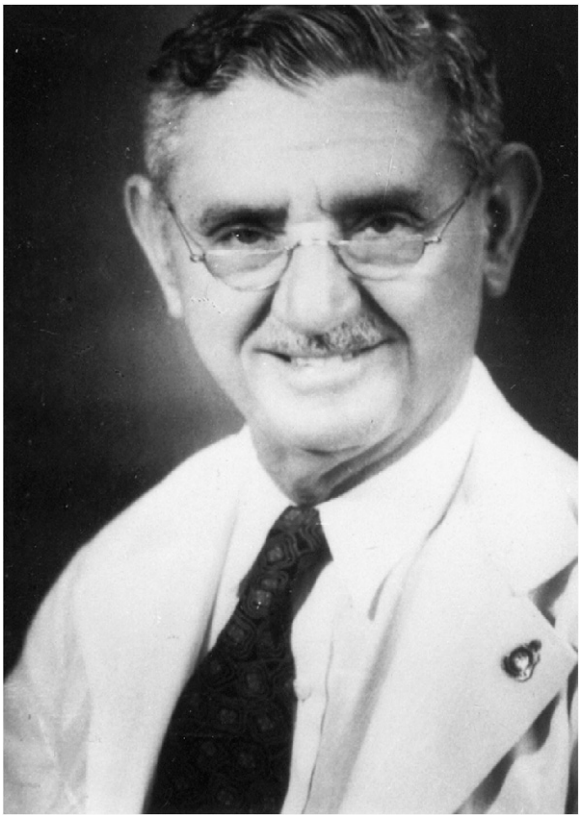

Fig. 5. Dr Hugo Flecker (1884–1957), former Melbourne radiologist and dermatologist who moved to Cairns in 1930. There he advanced the knowledge of Australian botany, zoology and toxinology. In 1956, the world's most venomous creature, the box jellyfish, *Chironex fleckeri*, was so named to honour his life and work. After Fenner (1990) and Fevers and Frontiers (Eds Pearn and Cobcroft, 1990) with acknowledgments.

## 8. The funnel-web antivenom

The development of the funnel-web spider antivenom stretched over decades and involved many of the key players in 20th century toxinology in Australia. Although the Sydney funnel web spider was first described in 1877 (Pickard-Cambridge), its fearsome toxicity was not formally recognised until the 1930s (Cleland, 1932). As in so many aspects of toxinology, it was Kellaway who undertook the first experiments on this venom and whose speculations on the nature of the venom proved prescient (Kellaway, 1934; Sutherland, 1983). Although further progress was made by Weiner in the 1950s (using thousands of spiders) (Weiner, 1957, 1959b, 1961), it was Sutherland whose dogged persistence was ultimately rewarded with successful 1981 release of the clinically tested antivenom (Sutherland, 1980). This long story is documented in detail elsewhere (Sutherland, 1983; Sutherland and Tibballs, 2001b) and is reviewed by others in this edition of the journal (Nicholson et al., 2006; Tibballs, 2006a).

## 9. Veterinary antivenoms—snake antivenoms

An estimated 6200 cases of snakebite are reported annually to veterinary clinics around Australia of which 78% of these occur in rural Australia and 22% in urban areas (Mirtschin et al., 1998). There are undoubtedly many more that are never reported to veterinary clinicians. Brown snakes (76%), tiger-type snakes (13%), and black snakes (6%) are the main species types responsible for significant bites (Mirtschin et al., 1998). Cats and dogs are the most frequently reported victims. It is estimated that, when antivenom is administered, 91% of cats and 75% of dogs survive whereas only 66% of cats and 31% of dogs survive without antivenom. Until recently, veterinary snake antivenoms were merely either human antivenoms used to treat animals or various versions of human antivenoms packaged and combined for animal use. Human antivenoms, especially out of date vials, are still used on occasion in animals.

In 1994, CSL Limited released the combined Tiger-Brown antivenom for veterinary use. This product was a recognition that this combination covered the majority of snakebite cases treated by

Australian veterinarians. In 2001, a newer formulation of this antivenom was released that contained 3000 units of Tiger snake antivenom and 1000 units of Brown snake antivenom (CSL Antivenoms, CSL Animal Health Brochure). A further change occurred in December 2003 when Pfizer announced that it had agreed to purchase CSL's Animal Health Division, including the right to sell veterinary antivenoms. Currently, Pfizer sells the brown snake (500 units per vial) and combined tiger/brown products (3000 and 1000 units, respectively, per vial) to Australian vets. Although several additional antivenom producers exist in Australia (discussed below), none sell products for use in humans against Australian snakes.

## 10. Tick antitoxin

Whilst Joseph Bancroft was the first to recognise the paralytic illness inflicted by the scrub tick (Bancroft, 1866), no progress was made on addressing this problem until the "highly practical" work of Ross (1975) (Fig. 6). One of the few Australian scientists recognised on our national currency (his head features opposite that of Howard Florey on the $50 note). Fortunately, for toxinology, this Sydney veterinary graduate failed in his 1925 attempt to establish a practice in the heart of Sydney, convincing him to spend most of the next decade on research into animal parasites at the veterinary school and the CSIR (Ross, 1975). Also fortunate for Australia was the fact that his father was correct (and survived!) when he chose to have "himself bitten by the supposedly deadly Australian Black snake in order to prove that its reputation was exaggerated" (Ross, 1975)! Driven by the needs of the pastoral industry, Ross focused his research on hydatid disease, the liver fluke and the scrub tick (*I. holocyclus*). Ross quickly demonstrated that extracts of the salivary glands of this arthropod, when injected into mice, produced a paralytic syndrome (Ross, 1926). Thereafter he developed methods for actively immunising dogs with short periods of attachment of an engorged female adult tick and showed that the serum of these "over-immunised" dogs could be used to treat this envenomation. Indeed CSIR reports suggest that 75% of poisoned dogs so treated recovered (Ross, 1935, 1975) (Fig. 5).

Based on this work, an "antitoxin" was released by CSL in 1938 (Oxer and Ricardo, 1942). Several decades later George Kaire of CSL was involved in efforts to improve the production of this product (Kaire, 1965, 1966). The characterisation of tick toxins and Kaire's role is further discussed elsewhere in this journal (Nicholson et al., 2006). One of these additional developments was the involvement of a subcontractor, "Supreme Serums" of Lismore, NSW, to provide the hyperimmune tick serum from dogs. This company started by a veterinarian, Dr. Keith Curtain, began producing tick antiserum in 1958 and is now run by his son John Curtain. This product was then, and still is, subsequently processed, by CSL in Melbourne, into the whole canine immunoglobulin antivenom for human use. This cooperative arrangement commenced in the early 1960s when CSL found it uneconomic undertake the whole process itself (the paralysis tick being endemic in to the Lismore region) (Dr. Keith Curtain, Supreme Serums, Pers. Commun., 2005).

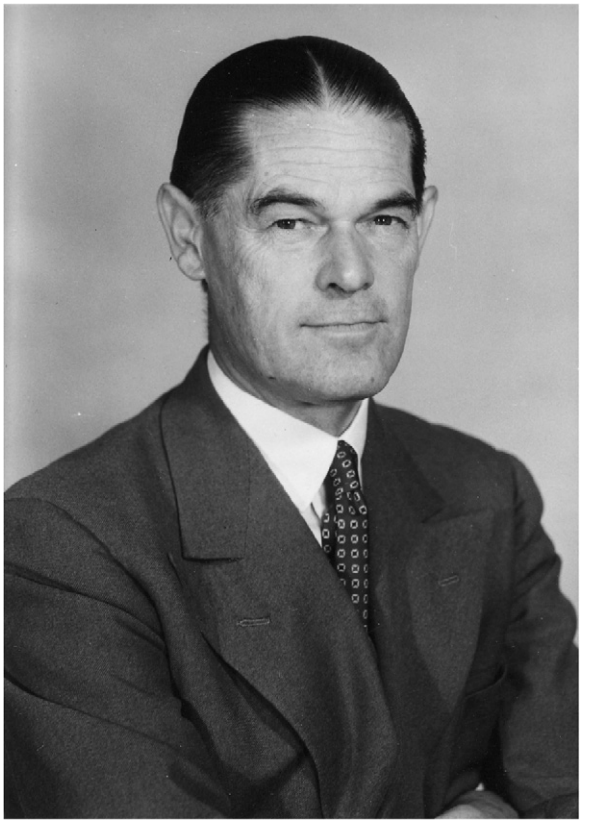

Fig. 6. Ian Clunies Ross (1899–1959), veterinarian, charismatic scientist, administrator and pioneer tick toxin researcher. Photograph by Division of Building Research, CSIRO, Australia, Negative 2473, reproduced with permission of Australian Academy of Science.

## 11. Other veterinary antivenoms

There are currently four producers of tick antivenoms for veterinary use. In addition to Supreme Serums, now known as "Summerland Serums" (which makes two tick antivenoms, one being more refined) (granted full licence 19/2/01, Commonwealth of Australia Gazette, 2001), the Australian Veterinary Serum Laboratories (AVSL) also in Lismore, established in 1968, began by producing for several veterinary clinics in northern NSW (National Registration Authority for Agricultural and Veterinary Chemicals, 2001). Over the intervening years both the product and the process developed with improvements in manufacture. A considerable colony of dogs had to be maintained year round although it was only possible to produce the *Ixodes* antivenom for 5 or 6 months of the year when the ticks were available.

In 1989, AVSL looked at the possibility of producing snake antivenoms from the dogs in the period of the year when they were not required for tick antivenom production. This involved the development of tests for the estimation of antibodies to black, brown and tiger snake venoms as well as research into the effectiveness of various adjuvants. Further work led to the successful production and registration of snake antivenom in dogs (a brown snake monovalent). Owing to economic considerations the initial production of antivenoms in dogs was substituted, firstly by a combination of production in both dogs and sheep, and then sheep only. Since 1997, the antibody levels of both black and tiger snake antisera have been increased considerably and more streamlined procedures for brown snake antivenom production developed. Work is currently in progress at AVSL towards the commercial production of both black and tiger snake antivenoms (the tiger snake product has now been approved and registered). Summerland Serums are awaiting approval of their two new veterinary antivenoms: a brown snake monovalent and a combined tiger/brown. The other two Lismore-based tick antivenom manufacturers (Warne and Webster Serum [Ixhit tick antitoxin serum registered 24/11/03, Australian Pesticides and Veterinary Medicines Authority, 2004a] and Northern Serums [granted full licence for tick antiserum 28/10/04, Australian Pesticides and Veterinary Medicines Authority, 2004b]) do not, at this stage, manufacture snake antivenoms.

## 12. Other antivenoms made in Australia

At various times from 1960 on, CSL explored the production of equine antivenoms for non-Australian snakes. The first such product, an antivenom for the Malayan pit viper (*A. rhodostoma*) found its way into clinical use, between 1961 and 1964, in Malaysia (Reid, 1980). In the early 1980s, another product was developed by CSL for sale in Malaysia, this time it was a cobra (*Naja* sp.) antivenom (Sutherland, 1998). Unlike the first product, an $(Fab)_2$ antivenom, the latter was unpurified, freeze-dried whole horse serum. Only a single batch of this antivenom was made (Sutherland, 1998).

More recently Australia has become the site of production of new US and European antivenoms. Protherics Inc (USA) is the sole licensed supplier of human therapeutic polyvalent rattlesnake antivenom, CroFab™, to the North American market. The product is processed by the parent company, Protherics PLC (UK), from hyper-immune ovine serum produced by its Australian subsidiary, Protherics Australasia Pty Ltd. The company history traces back to the early 1980s when a company, Polyclonal Antibodies Limited, was formed to produce antibodies from sheep for diagnostic purposes and recognised the opportunity to produce therapeutic antivenom products in the same manner. A new company was formed, TAb Inc, UK based but American owned, and pursued the development of a number of antivenom products, in particular a new crotalid antivenin, but also antivenoms for *Vipera berus* in Europe, *Echis ocellatus* in Nigeria, and *Daboia Russelli pulchella* in Sri Lanka. After a name change to Therapeutic Antibodies Inc and then a merger with another UK company, ownership of the company returned to the UK as Protherics PLC and only the *Crotalid* and *Vipera* products remain within the company today.

Why sheep, and why in Australia? Ovine antibodies were chosen as they are less allergenic in humans than equine IgG and sheep are abundantly available and relatively easy to manage. In the early 1990s it was recognised that sheep used for antivenom production needed to be located in a clean environment, free of serious viral diseases and particularly free of the transmissible spongioform encephalopathy disease "Scrapie", an environment that only Australia and New Zealand can provide. Hence a project was started with the Institute of Medical and Veterinary Science in Adelaide that

eventually became the company Tab Australia Pty. Ltd and ultimately Protherics Australasia, as it is today. Protherics Australasia now runs a hyper-immune sheep flock of around 4000 head at Mintaro in the mid north of South Australia with the majority of the sheep involved in the production of CroFab™ but Protherics also produces antivenom for European viper bites (ViperaTAB) and an antidote for treatment of Digoxin drug toxicities (DigiFab™).

All Protherics products are produced on a platform of technology involving separation of the IgG from hyper-immune sera, digestion to release Fab fragments and affinity purification of the final product. A Fab product is preferred over IgG or $F(ab)_2$ largely for its patient safety profile. The use of ovine serum, the absence of the Fc antibody component, and the small Fab molecule give Protherics' products a very significantly lower incidence of anaphylaxis compared with alternative equine derived products. However, in contrast to the experience with equine $(Fab)_2$ antivenoms, recurrence of coagulopathy has proved problematic. This improved safety profile has resulted in a much greater acceptance of the use of an antivenom by treating physicians in the US and the number of CroFab™ treatments now used for viper bites in the USA is 3–4x higher than that of previously available Wyeth equine derived crotalid polyvalent antivenom (Dart and McNally, 2001).

CroFab™ is prepared as a polyvalent product comprising individual components generated against the venoms of *Crotalus atrox*, *C. adamanteus*, *C. scutulatus* and *Agkistrodon piscivorus*. The spectrum of venom components across these four species provides a sufficiently wide spectrum of antibodies to neutralise the normal range of viper bites that occur in North America. Although process improvements and technological developments, both past and future will enable a significant reduction in production costs, the high cost of regulatory approval relative to the size of the market remains a barrier to expanding Protherics' technology into the production of antivenoms for many other markets, as it does for all antivenom manufacturers.

## 13. Other experimental antivenoms

In the early 1990s experimental studies began to suggest that there had been an underestimation of the amounts of antivenom required to neutralise the procoagulants of certain Australia snake venoms. Tibballs and Sutherland (1991) found that the doses of CSL Ltd brown snake antivenom required to prevent severe cardiovascular depression and coagulopathy induced by *Pseudonaja textilis* and *Pseudonaja affinis* venoms in dogs was 25x and 10x respectively the recommended dose for clinical use. Subsequently, Sprivulis et al. (1996) undertook in vitro testing of both canine and human plasma and *N. scutatus*, *P. textilis*, *P. nuchalis* and *P. affinis* venoms. They observed that between 10x and 20x the expected dose of the relevant antivenom was required to neutralise the procoagulant actions of brown and tiger snake venoms.

Further Masci et al. (1998) reported that CSL brown snake antivenom had significantly lower avidity for the prothrombin activator in *P. textilis* venom than other venom components, including the neurotoxins. Moreover it became clear that neutralization of the prothrombin activator was time dependent and that 40% remained unneutralised after 40 min incubation. With apparent deficiencies in two CSL antivenoms being reported in the 1990s, work commenced in a collaborative project involving two companies, Venom Science Pty Ltd and Venom Supplies Pty Ltd and the Institute of Medical and Veterinary Science to design antivenoms that addressed this problem. Because of the costs involved with producing human antivenoms and the difficulties in obtaining sufficient clinical trial data required to get the product approved through the human regulatory authority (the Therapeutic Goods Administration), it was decided to focus on a veterinary antivenom first. Priority was given to the production of a brown snake (*Pseudonaja* genus) antivenom because of their leading importance of this envenomation to animal and veterinary medicine in Australian (Sutherland and Leonard, 1995; Mirtschin et al., 1998).

Work commenced in 1992 to produce a brown snake antivenom using hen egg yolk (IgY) antibodies against brown snake venom components (Madaras et al., 2005). After 2 years a brown snake antivenom was produced, tested and trialed with three veterinary clinics in South Australia. This antivenom compared favourably with CSL Brown snake antivenom. However, it soon became apparent that the IgY serum was deficient in antibodies directed against the low molecular weight, post-synaptic neurotoxins in brown snake venom. Therefore, it was decided to make a complementary ovine brown snake antivenom (Madaras et al., 2005). The

resultant combined avian IgY and ovine IgG antivenom took until 2001 to develop. This antivenom was tested using immunoelectrophoresis to ensure all major antigens were being recognised by antibodies, a clotting test to ensure the procoagulant was being bound by antibodies as well as a mouse protection test. The latter largely examines the neurotoxic effect of the venom. The results showed a remarkable improvement in procoagulant neutralisation by the new antivenom when compared with the CSL product. It was many times more efficacious, compared with CSL brown snake antivenom against this enzyme, with equivalent or better performance in the mouse protection test (Madaras et al., 2005). However, as registration could not be justified against the projected small return this antivenom project was terminated. It should be noted that recent data suggests that, at clinically relevant venom concentrations, the CSL brown snake antivenom may be effective at neutralising the brown snake prothrombin activator (Isbister et al., 2006).

## 14. Poisonous plants

Studies of snake envenomation fell somewhat into abeyance for almost three decades after Martin's and Tidwell's experiments in the decade following 1896. This era, however, saw a blossoming of Australian plant toxinology, in turn building on the encyclopaedic work of Ferdinand von Mueller (1825–96) (Churchill et al., 1984). The cognate study of medicinal and toxic flora appeared in a miscellany of diverse publications with titles ranging from such themes as Medicinal Plants in Flower-farming for Perfumes and Medicines (Dunnicliff, 1892) to Indigenous vegetable drugs (Maiden, 1899). These included detailed research studies to understand the antiseptic, toxic and hoped-for medicinal properties of such genera as Eucalyptus (Lauterer, 1895; Baker and Smith, 1920), and Acacia (Pearn, 1993).

In Australia, the three most significant plant groups which are potentially lethal if ingested are indigenous fungi and the introduced pink or white oleander, *Nerium* oleander; and the yellow oleander, *Thevetia peruviana* (Pearn, 1987). Professor J.B. Cleland published the Toadstools and Mushrooms and other large Fungi of South Australia in 1935 (Cleland, 1935), which became a pioneering reference publication for toxic fungi in southern Australia. Non-fatal but serious medical consequences which followed the accidental or suicidal ingestion of other toxic flora included contact with the astringent properties of native Euphorbia. Such were due to toxic saponins and capsaicnoids (de Vries and Blumberg, 1989).

The 1920s saw the establishment of inter-disciplinary co-operative committees to review and document the effects of Australian toxic flora. Several States, including NSW and later Queensland, established Poison Plants Committees to monitor the toxic effects of both indigenous and introduced flora which were harmful to stock or humans (Finnemore, 1929). A great array of cyanogenetic glycosides, tannins, toxic alkaloids and toxic saponins were described in indigenous flora, in the first three decades after Federation. Professor J.B. Cleland, of Adelaide, undertook early feeding experiments of putative toxic native Australian plants, including studies of *Indigofera australis* (Cleland, 1914). This genus was of special interest as it later proved to be one of the first examples of mammalian food-chain toxicity. It was subsequently found that wild horses which had eaten *Indigofera* spp. in central Australia, and subsequently had developed degenerative peripheral neurological signs (''Birdsville Horse Disease''), were killed and used as food for local working dogs, which in turn also developed acute neurotoxicity (Pearn, 1967a, b). The further contributions of Cleland to Australian toxinology are described elsewhere (Southcott, 1972).

## 15. Conclusion

In the rush of the 21st century genome-era it is all too easy to discount the value of the pioneer toxinologists described herein, whose equipment was rudimentary and resources were few. However, these factors meant that their vision was just as keen as those enriched by contemporary technology. This survey of the domain on 20th century toxinology in Australia reflects the acuity of their observations and how we daily see further as a consequence of their contributions. JB Cleland (in Cleland and Southcott, 1965) put it thus: ''They that went down to the sea in ships in the leisurely days of sail had time to spare for the study of the creatures of the deep. The medical man of that time was often a keen naturalist…The present monograph…is not so much a continuation of the series as a product on its own — to be followed, it is hoped, by other accounts along similar lines contributed by younger

scientifically trained men who may like perhaps to remember that one whose upbringing was essentially in the Victorian Era was associated with the first of the series".

## Acknowledgements

We thank Mr. Rob Mugford (Protherics, Turretfield, South Australia), Dr. Keith Curtain and Mr. John Curtain (Lismore Supreme Serums and Summerland Serums, NSW) and Mr David Jones (Australian Veterinary Serum Laboratory, Lismore, NSW) for their assistance on aspects of the manuscript. The Australian Venom Research Unit gratefully acknowledges funding support from the Australia Government Department of Health and Ageing and Snowy Nominees and the University of Melbourne for a History of the University Study Grant. We thank Ms Christine Woollett, Section Co-ordinator, Library and Information Services, The Royal Society, London, and Ms Sharon Abrahams, Project Officer, Australian Academy of Science, Canberra, for the provision of photographs and Mr Peter Hobbins for additional research on Charles Kellaway.

## References

Australian Pesticides and Veterinary Medicines Authority, 2004a. Product Name: Ixhit Paralysis Tick Antitoxin Serum. Gazette APVMA, vol. 1, p. 6.

Australian Pesticides and Veterinary Medicines Authority, 2004b. New Licences: Northern Serums Pty Ltd, Gazette APVMA, vol. 12, p. 67.

Baker, R.T., Smith, H.G., 1920. A Research on the Eucalyptus. Especially in Regard to their Essential Oils, 2nd ed. Technical Museum [Technical Education Series 24], Sydney, pp. 96–97.

Bancroft, T.L., 1893. Australas. Med. Gaz. 12, 43–45.

Bancroft, J., 1866. Poisonous Animals [Publisher and Place unknown].

Bancroft, T.L., 1894. Some further observations on the physiological action of snake venom, together with a reference to the strychnine cure of snakebite. Australas. Med. Gaz. 13, 228–230.

Baxter, E.H., Gallichio, H.A., 1974. Cross-neutralisation by tiger snake (*Notechis scutatus*) antivenene and sea snake (*Enhydrina schistosa*) antivenene against several sea snake venoms. Toxicon 12, 273–278.

Baxter, E.H., Marr, W.R., 1970. Recent investigations on sea-wasp stingings in Australia. Med. J. Aust. 1, 508.

Beadnell, C.E., Rider, T.A., Williamson, J.A., Fenner, P.J., 1992. Management of a major box jellyfish (*Chironex fleckeri*) sting. Lessons from the first minutes and hours. Med. J. Aust. 156, 655–658.

Bill, G., 1902. Notes on a case of snake-bite treated with antivenine. Intercolon. Med. J. Aust 1, 346.

Boyd, J., 1966. Neil Hamilton Fairley, 1891–1966. Biog. Mem. R. Soc. 12, 122–145.

Brogan, A.H., 1990. Chapter 16: Research and Development. In: Committed to Saving Lives—A History of the Commonwealth Serum Laboratories, Hyland House, Melbourne, pp. 207–231.

Burnet, M., 1971a. The Origins of the Institute. Walter and Elisa Hall Institute 1915–1965, Melbourne University Press, pp. 3–15 (Chapter 1).

Burnet, M., 1971b. The First Director. Walter and Elisa Hall Institute 1915–1965, Melbourne University Press, pp. 16–20 (Chapter 2).

Burnet, M., 1971c. Kellaway as Director. Walter and Elisa Hall Institute 1915–1965. Melbourne University Press, pp. 21–32 (Chapter 3).

Burnet, M., 1971d. Poisons. Walter and Elisa Hall Institute 1915–1965. Melbourne University Press, pp. 90–101 (Chapter 8).

Cann, J., 1986. Recognized treatment of snake bite. In: Snakes Alive. Snake Experts and Antidote Sellers of Australia. Kangaroo Press Pty Ltd, Kenthurst NSW Australia, p 49 (Chapter 2).

Calmette, A., 1894. Propriétés du serum des animaux immunises contre le venin des serpents; therapeutique de l'envenimation. CR Acad. Sci. (Paris) 118, 720–722.

Chetty, N., Du, A., Hodgson, W.C., Winkel, Fry, B.G., 2004. The in vitro neuromuscular activity of Indo-Pacific sea-snake venoms: efficacy of two commercially available antivenoms. Toxicon 44, 193–200.

Chick, H., 1956. Charles James Martin 1866–1955. Biog. Mem. R. Soc. 2, 173–208.

Churchill, D.M., Muir, T.B., Sinkora, D.M., 1984. The published works of Ferdinand J.H. Mueller (1825–1896). Suppl. Muelleria 5, 229–248.

Cleland, J.B., 1914. Experimental feeding with some alleged poison plants of New South Wales. Agric. Gaz. NSW 25, 65.

Cleland, J.B., 1932. Injuries and diseases in Australia attributable to animals (other than insects). Med. J. Aust. 1, 157–166.

Cleland, J.B., 1935. Toadstool and Mushrooms and Other Larger Fungi of South Australia. Parts I and II. A.B. James (Govt Printer), Adelaide.

Cleland, J.B., Southcott, R.V., 1965. Injuries to man from marine invertebrates in the Australian region. Canberra: National Health and Medical Research Council. Special Report Series No. 12.

Commonwealth of Australia (Agricultural and Veterinary Chemicals Code Act 1994), 2001. New licences: Lismore Supreme Serums. Anti-tick serums. Gazette 4, 15.

CSL, 2001. Taipan antivenom product information. CSL Limited package insert Aust R74898.

Currie, B., 1994. Clinical implications of research on the box-jellyfish *Chironex fleckeri*. Toxicon 32, 1305–1313.

Dale, H.H., 1953. Obituary notice: C.H. Kellaway. Obit. Notices Fellows R. Soc. 8, 503–521.

Dart, R.C., McNally, J., 2001. Efficacy, safety, and use of snake antivenoms in the United States. Ann. Emerg. Med. 37, 181–188.

De Vries, D.J., Blumberg, P.M., 1989. Resiniferatoxin (RTX): a novel and extremely potent capsaicinoid from plants of the genus Euphorbia. Life Sci. 44, 711–715.

Dunnicliff, A.A., 1892. Medicinal plants. In: Flower-farming for Perfumes and Medicines. Agric. Gaz. NSW. 3, 954–961.

Fairley, N.H., 1929a. The dentition and biting mechanism of Australian snakes. Med. J. Aust. 1, 313–327.

Fairley, N.H., 1929b. Criteria for determing the efficacy of ligature in snake bite (the subcutaneous-intravenous Index). Med. J. Aust. 1, 377–394.

Fairley, N.H., 1929c. The present position of snake bite and the snake bitten in Australia. Med. J. Aust. 1, 296–313.

Fairley, N.H., Splatt, B., 1929. Venom yields in australian poisonous snakes. Med. J. Aust. 1, 336–348.

Feldberg, W., Kellaway, C.H., 1937. Liberation of histamine from the perfused lung by snake venoms. J. Physiol. 90, 257–259.

Feldberg, W., Kellaway, C.H., 1938. Liberation of histamine and formation of lysocithin-like substances by cobra venom. J. Physiol. 94, 187–226.

Fenner, P., 1990. Medical frontiers in Australian Seas. In: Pearn, J., Cobcroft, M. (Eds.), Fevers and Frontiers. Amphion Press, Brisbane, p. 78 (Chapter 3).

Finnemore, H., 1929. Report from the Chairman, The Poison Plants Committee [of N.S.W.]. J. Council Sci. Indus. Res. [Org] 2, 40–48.

Hawgood, B.J., 1992. Pioneers of anti-venomous serotherapy: Dr. Vital Brazil (1865–1950). Toxicon 30, 573–579.

Hawgood, B.J., 1997. Sir Charles James Martin MB FRS: Australian Serpents and Indian plague, one hundred years ago. Toxicon 35, 999–1010.

Hawgood, B.J., 1998. Hugh Alistair Reid OBE MD: investigation and treatment of snake bite. Toxicon 36, 431–446.

Hawgood, B.J., 2006. The marine biologist. Bob Endean. Toxicon, in press.

Isbister, G.K., O'Leary, M.A., Schneider, J.J., Brown, S.G.A., Currie, B.J., 2006. Efficacy of antivenom against the procoagulant effect of Australian brown snake (*Pseudonaja* spp.) venom: in vivo and in vitro studies. Toxicon, in press.

Kaire, G.H., 1964. Sea-snake antivenom. Med. J. Aust. 2, 729.

Kaire, G.H., 1965. A method for assay of canine anti-tick serum. Aust. Vet. J. 41, 239.

Kaire, G.H., 1966. Isolation of tick paralysis toxin from *Ixodes holocyclus*. Toxicon. 4, 91–97.

Kellaway, C.H., 1934. A note on the venom of the Sydney funnel-web spider *Atrax robustus*. Med. J. Aust. 1, 678–679.

Kellaway, C.H., 1937a. Snake venoms I. Their constitution and therapeutic applications. Bull. Johns Hopkins Hosp. 1, 1–17.

Kellaway, C.H., 1937b. Snake venoms II. Their peripheral action. Bull. Johns Hopkins Hosp. 1, 18–39.

Kellaway, C.H., 1937c. Snake venoms III. Immunity. Bull. Johns Hopkins Hosp. 3, 159–177.

Kellaway, C.H., 1938. The symptomatology and treatment of the bites of Australian snakes. Med. J. Aust. 2, 585.

Kellaway, C.H., 1939. Animal poisons. Ann. Rev. Biochem. 8, 541–556.

Kellaway, C.H., Burnet, M., 1930. Recent work on staphylococcal toxins with special reference to the interpretation of the Bundaberg fatalities. Med. J. Aust. 2, 95.

Kellaway, C.H., Burnet, M., Williams, F.E., 1930. The pharmacological action of the exotoxin of *staphylococcus aureus*. J. Path. Bact. 33, 889.

Kellaway, C.H., Dale, H.H., 1921. Anaphylaxis and anaphylatoxins. Phil. Trans. B. 211, 273.

Koch, L., 1871–1877. Die Alachniden Australiens, Nach der Natur Beschrieben und Abgebildet. Bauer & Raspe, Nuremberg.

Krefft, G., 1869. The Snakes of Australia: An Illustrated and Descriptive Catalogue of All The Known Species. Government Printer, Sydney.

Lauterer, J., 1895. Queensland native astringent medicines, illustrated by the chemistry of the gums of eucalyptus and angophoras. Australas. Assoc. Adv. Sci. 6, 293–304.

MacIntyre, S., Selleck, R.J.W., 2003. A Short History of the University of Melbourne. Melbourne University Press, Melbourne (p. 153).

Madaras, F., Mirtschin, P.J., Kuchel, T., 2005. Antivenom development in Australia. Toxin Rev. 24, 79–94.

Maiden, J.H., 1899. Indigenous vegetable drugs. Part II. Agric. Gaz. NSW 10, 117–130.

Martin, C.J., 1892. Observations of the poisonous constituents of the venom of the Australian Black Snake (*Pseudechis porphyriacus*). Proc. Linn. Soc. NSW 7, 319–322.

Martin, C.J., 1895. On the physiological action of the venom of the Australian Black Snake (*Pseudechis porphyriacus*). J. Roy. Soc. NSW. 29, 146.

Martin, C.J., 1896. Note on a method of separating colloids from crystalloids by filtration. An explanation of the marked different in the effect produced by subcutaneous and intravenous injection of the venom of Australian snakes. J. R. Soc. NSW 30, 147.

Martin, C.J., 1897a. The curative value of Calmette's anti-venomous serum in the treatment of inoculations with the poisons of Australian snakes. Intercolon. Med. J. Australas. 2, 527–536.

Martin, C.J., 1897b. On the advisability of administering curative serum by intravenous injection. Intercolon. Med. J. Australas. 2, 537–538.

Martin, C.J., 1898. A rejoiner to M. le Dr Calmette. Intercolon. Med. J. Australas. 3, 197.

Martin, C.J., Hill, J.P., 1894. On a platypus embryo from an intra-uterine egg. Proc. Linn. Soc. NSW 9, 738.

Martin, C.J., Smith, J.M., 1892. The venom of the Australian black snake (*Pseudechis porphyriacus*). Proc. R. Soc. NSW 26, 240.

Masci, P.P., Mirtschin, P.J., Nias, T.N., Turnbull, R.K., Kuchel, T.R., Whitaker, A.N., 1998. Brown snakes (*Pseudonaja* genus): venom yields, prothrombin activator neutralization and implications affecting antivenom usage. Anaesth. Intensive Care 26, 276–281.

Mirtschin. P.J., 2006. The pioneers of venom production for antivenoms in Australia. Toxicon, in press.

Mirtschin, P.J., Masci, P., Paton, D.C., Kuchel, T., 1998. Snake bites recorded by veterinary practices in Australia. Aust. Vet. J. 76, 195–198.

von Mueller, F.J.H., 1858–1882. Fragmenta Phytographiae Australiae [Published in Twelve Parts I–XII] [Facsimile Editions 1974]. A. Asher and Co., Amsterdam.

National Registration Authority for Agricultural and Veterinary Chemicals, 2001. Australian manufacturers of veterinary chemical products as on 31 December 2001, Australian Veterinary Serum Laboratories, 6.

Nicholson, G.M., Graudins, A., Wilson, H.I., Little, M., Broady, K.W., 2006. Arachnid toxinology in Australia: from clinical toxicology to potential applications. Toxicon, in press.

Oxer, D.T., Ricardo, C.L., 1942. Notes on the biology, toxicity and breeding of Ixodes holocyclus (Neumann). Aust. Vet. J. 18, 194–199.

Pearn, J., 1994. Hugo Flecker's contributions to medicine and natural history. Med. J. Aust. 161, 63–70.
Pearn, J.H., 1967a. Report of a new site-specific cleft palate teratogen. Nature. 215, 980–981.
Pearn, J.H., 1967b. Studies on a site-specific cleft palate teratogen The toxic extract from *Indigofera spicata* Forssk. Br. J. Exp. Pathol. 48, 620–626.
Pasteur, L., 1881. De l'atténuation des virus et de leur retour à la virulence. CR Acad. Sci. Agric. Bulg. 92, 429–435.
Pearn, J.H., 1987. Oleander poisoning. In: Toxic Plants and Animals: A Guide for Australia. The Queensland Museum, Brisbane, pp. 37–49 (Chapter 4).
Pearn, J.H., 1993. Acacias and Aesculapius. Australian native wattles and the doctors they commemorate. Med. J. Aust. 159, 729–738.
Pearn, J.H., 1994a. Around the Rim. The role of surgeons in discovery and research in the Pacific Rim. Part I. Surgeons in the Pacific—expeditioners and expedition leaders. Aust. N. Z. J. Surg. 64, 38–44.
Pearn, J.H., 1994b. Around the Rim. The role of surgeons in discovery and research in the Pacific Rim. Part II. Surgeons in the Pacific—colonists and scientists. Aust. N. Z. J. Surg. 64, 121–124.
Pearn, J.H., 2001. Introduction. In: A Doctor in the Garden. Amphion Press, Brisbane, p. 4.
Pearn, J.H., 2004. Medicine at war—The "pivot years" of 1943 and 1944 in the New Guinea campaign. In: Dennis, P. (Ed.), Foundations of Victory: The Pacific War 1943–1944". Australian Defence Force Academy, Canberra.
Pearn, J.H., 2006. The medical zoologist. Ronald Vernon Southcott. Toxicon, in press.
Pearn, J.H., Fenner, P.J., 2006. The jellyfish hunter: Jack Barnes. A pioneer medical toxinologist in Australia. Toxicon, in press.
Pearn J.H., Winkel, K.D., 2006. Toxinology in Australia's colonial era: a chronology and perspective of human envenomation in nineteenth century Australia. Toxicon, in press.
Reid, H.A., 1962. Sea-snake antivenene: successful trial. Br. Med. J. II, 576–579.
Reid, H.A., 1980. Antivenom reactions and efficacy. Lancet, 1024–1025.
Ross, I.C., 1926. An experimental study of tick paralysis in Australia. Parasitology 18, 410–429.
Ross, I.C., 1935. A fatal disease of dogs and other animals in eastern Australia. J. Council Sci. Ind. Res. Aust. 8, 8–13.
Ross, A.I.C., 1975. Ian Clunies Ross 1899–1959. Rec. Aust. Acad. Sci. 3, 84–108.
Sewall, H., 1887. Experiments on the preventive inoculation of rattlesnake venom. J. Physiol. 8, 203–210.
Sprivulis, P., Jelinek, G.A., Marshall, L., 1996. Efficacy and potency of antivenoms in neutralizing the procoagulant effects of Australian snake in dog and human plasma. Anaesth. Intens. Care 24, 379–381.
Southcott, R.V., 1972. John Burton Cleland. Med. J. Aust. 1, 192–193.
Sutherland, S.K., 1980. Antivenom to the venom of the male Sydney funnel-web spider *Atrax robustus*. Preliminary report. Med. J. Aust. 2, 437–441.
Sutherland, S.K., 1983. Genus *Atrax* Cambridge, the funnel-web spiders. In: Australian Animal Toxins: The Creatures, Their Toxins and Care of the Poisoned Patient. Oxford University Press, Melbourne, pp. 255–298.
Sutherland, S.K., 1994. Antivenom research in Australia. Med. J. Aust. 161, 48–50.
Sutherland, S.K., 1998. A Venomous Life. Hyland House, Melbourne (p. 266).
Sutherland, S.K., Leonard, R.L., 1995. Snakebite fatalities and a management update. Med. J. Aust. 163, 616–618.
Sutherland, S.K., Tibballs, J., 2001a. Genus *Pseudechis*, Black snakes. In: Australian Animal Toxins: The Creatures, Their Toxins and Care of the Poisoned Patient. Oxford University Press, Melbourne, pp.159.
Sutherland, S.K., Tibballs, J., 2001b. The genera *Atrax* and *Hadronyche*, funnel-web spiders. In: Australian Animal Toxins: The Creatures, Their Toxins and Care of the Poisoned Patient. Oxford University Press, Melbourne, pp. 402–464.
Tibballs, J., 2006a. Struan Sutherland—Doyen of envenomation in Australia. Toxicon, in press.
Tibballs, J., 2006b. Jellyfish venoms, toxins and envenomation syndromes. Toxicon, in press.
Tidswell, F., 1899. Report on Protective Inoculation Against Tick Fever: An Account of An Experimental Inquiry Into Its Effect On Cattle, And On Meat And Milk; Together With Some Notes On Protective Measures Other Than Inoculation. Government Printer, Sydney.
Tidswell, F., 1900. Second Report On Protective Inoculation Against Tick Fever: An Account Of An Experimental Inquiry Concerning The Protective Value Of Inoculation; Together With Some Observations Upon Cattle Ticks And Upon The Practice Of Inoculation. Government Printer, Sydney.
Tidswell, F., 1902. A preliminary note on the serum therapy of snake-bite. Australas. Med. Gaz. 21, 177–181.
Tidswell, F., 1906. Researches on Australian Venoms Snake-Bite, Snake-Venom and Antivenine, The Poison of The Platypus, The Poison of The Red-Spotted Spider. NSW Department of Public Health, Sydney.
Tisdall, H.T., Sewell, J.E., 1931. Treatment with antivenene twenty-four hours after a bite by a tiger snake. Med. J. Aust. 1, 604–605.
Tibballs, J., Sutherland, S.K., 1991. The efficacy of antivenom in prevention of cardiovascular depression and coagulopathy induced by brown snake (*Pseudonaja*) species venom. Anaesth. Intens. Care 19, 530–534.
Weiner, S., 1956a. The Australian red back spider (*Latrodectus hasselti*). I. Preparation of antiserum by the use of venoms absorbed on aluminium phosphate. Med. J. Aust. 1, 739–742.
Weiner, S., 1956b. The Australian red back spider (*Latrodectus hasselti*). II. Effect of temperature on the toxicity of venom. Med. J. Aust. 2, 331–334.
Weiner, S., 1957. The Sydney funnel-web spider (*Atrax robustus*) I. Collection of venom and its toxicity in animals. Med. J. Aust. 2, 377–382.
Weiner, S., 1959a. Observations on the venom of the Stonefish (*Synanceja trachynis*). Med. J. Aust. 1, 620–627.
Weiner, S., 1959b. The Sydney funnel-web spider (*Atrax robustus*) II. Venom yields and other characteristics of spiders in captivity. Med. J. Aust. 2, 679–682.
Weiner, S., 1959c. The production and assay of Stonefish antivenene. Med. J. Aust. 2, 715–719.
Weiner, S., 1960. Active immunisation of man against the venom of the Australian tiger snake (*Notechis scutatus*). Am. J. Trop. Med. Hyg. 9, 384–392.
Weiner, S., 1961. Observations on the venom of the Sydney funnel-web spider (*Atrax robustus*). Med. J. Aust. 2, 693–699.

Weiner, S., 2003. Latrodectism: a prospective cohort study of bites by formally identified redback spiders. Med. J. Aust. 179, 455.

Weiner, S., Drummond, F.H., 1956. Assay of spider venom and antivenene in Drosophila. Nature 178, 267–268.

Winkel, K., Hawdon, G., Fenner, P.J., Gershwin, L.A., Collins, A.G., Tibballs, J., 2003. Jellyfish Antivenoms: Past, Present and Future. J. Toxicol-Toxin. Rev. 22, 13–25.

Wood, I.J., 1984. Discovery and Healing in Peace and War. Acton Graphics, Melbourne (p. 15).