BIOGRAPHICAL MEMOIRS

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Sylvia Agnes Sophia Tait was born on 8 January 1917 in Tumen, Siberia, Russia. She was the daughter of James Wardropper, an agronomist and trader, working in Russia. It seems that James Wardropper worked there with his elder brother, Robert (Huntford 1997). The wife of James Wardropper, Ludmilla, was a Russian who had the rare distinction of graduating in mathematics from the University of Moscow in the time of the reign of the Tsar. James and Ludmilla Wardropper adopted a Russian girl, Pasha; she became part of the family and helped to look after Sylvia. During the revolution, in 1920 the whole family, including Pasha (but not including Robert) left Russia from Vladivostok for the UK, where James Wardropper eventually became a successful civil engineer. The fate of Robert Wardropper remains a mystery. The other Wardroppers first stayed in the UK in Ealing, London, where Sylvia attended the local secondary school, the Ealing County School for Girls. In her senior years there, she mainly studied languages, particularly German but also French and Latin. The Wardroppers had relatives in Germany and, before World War II, Sylvia spent some time in Germany, including Berlin, which improved her German. In addition, of course, at that time she spoke fairly fluent Russian with her mother and step-sister, Pasha. Sylvia had considerable trouble in establishing her citizenship because of her birthplace but eventually was officially declared British. Because of the nature of the father's history as a Scottish engineer in Russia and also the effects of the revolution, Sylvia never met her maternal grandparents and knew little about them.

Sylvia was quite a keen netball player at school until she tore a knee cartilage. This troubled her for the rest of her life and involved her in several fairly serious operations with three knee replacements. After leaving school, she had a short period at King's College, London, taking mainly courses in the German Department. However, she soon transferred to University

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College London where, after first qualifying in courses on science subjects, she eventually took a second-class honours degree in zoology in 1939, which was a worthy achievement in view of her earlier specialization in languages. In 1940 she married Flight-Lieutenant Anthony Simpson, a fellow student at University College, who then flew in the RAF Coastal Command. After winning a DFC, he was subsequently killed in action near Bergen, Norway, in 1941. Professionally, Sylvia took his name of Simpson (rather than her maiden name of Wardropper) until she married J. F. Tait in 1956. Soon after the death of her first husband, Sylvia A. S. Simpson joined the team of J. Z. Young (FRS 1945) in Oxford, who were working on nerve regeneration (1)*. In the same building, P. B. (later Sir Peter) Medawar (FRS 1949), who had graduated under J. Z. Young, was starting his well-known work on transplantation immunity for the MRC.

In 1944, after about three years in Oxford, Sylvia Simpson took up a more permanent position in the Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, UK, as an assistant to P. C. Williams, who was then Head of the Biological Unit on the fifth floor, which included the animal house. As a war effort, a Courtauld team, including Sylvia Simpson, E. C. (later Sir Charles) Dodds FRS, W. Lawson and P. C. Williams, tested synthetic analgesics as alternatives for opiates (2). She also worked with Williams and a chemist, A. E. Wilder-Smith, mainly on oestrogens (3–5, 7, 8). Later, Sylvia Simpson took over from Williams when he retired. Incidentally, Hans Selye, who had proposed the adaptation syndrome with a crucial role for a then unknown adrenal mineralocorticoid (Selye & Horava 1953), had been a visitor in the department and had taught Williams various surgical techniques, such as hypophysectomy. The animal house in the Courtauld Institute was very well equipped with surgical, rat breeding and constant-temperature rooms and a highly competent team of technicians. This was due largely to the reliance of the work of Dodds and W. Lawson, a chemist, on stilboestrol in the Courtauld Institute on reliable oestrogen bioassays (Dickens 1975). Later, this work was performed in collaboration with Sir Robert Robinson FRS at Oxford University (Dickens 1975). Sylvia Simpson did not take part in the original work on stilboestrol but the Courtauld Animal House continued to perform bioassays of oestrogen, usually under her supervision. This included a bioassay of synthetic oestrogens other than stilboestrol, including the isolation and identification of genistein, an oestrogen in clover in Australia (6). Genistein, an isoflavone, was another non-steroidal oestrogen. Incidentally, this compound, an undesirable abortificant in Australian sheep, has recently been found in soya beans and to inhibit prostate growth in mice. It has been suggested that the low rate of cancer of the prostate in Japanese men is due to the relatively high quantities of isoflavones in the diet. The bioassay of material from extracts of the clover, supervised by Sylvia Simpson, played a vital role in this successful pioneering work on genistein in the UK. The early period of her career in the Courtauld coincided with the assault on London by V1 and V2 rockets (the first V2 exploded nearby in Tottenham Court Road). Sylvia took a full part in air-raid duties, as did Dodds (Ranger 1985). The animal house in the Courtauld was particularly vulnerable during this time.

In 1948 a clinician, B. Lewis (known as ‘Bruin Lewis’), in the Department of Medicine, Middlesex Hospital, read a paper by Dorfman *et al*. (1947), which proposed a bioassay for adrenal mineralocorticoids (using deoxycorticosterone as a model steroid) from effects on the

* Numbers in this form refer to the bibliography at the end of the text.

urinary excretion of ^{24}Na in adrenalectomized rats. Lewis thought it would be useful for such a bioassay to be developed and applied in the Middlesex. He therefore separately approached Sylvia Simpson and a medical physicist in the Physics Department, James Tait (FRS 1959), to suggest that they collaborate to achieve this. As previously described, Sylvia Simpson was an experienced bioassayist seeking to broaden her work from the use of oestrogen assays. Tait was in charge of the use of radioactive isotopes in the Middlesex and was interested in expanding their application there. They therefore both accepted the suggested project and started to collaborate to develop the initial studies of Dorfman *et al*. (1947). This turned out to be a demanding task, particularly in view of their extensive routine duties. Incidentally, by an extraordinary coincidence, during this work it emerged that the father and grandfather of James Tait, marine engineers, were interned in Russia (in Odessa) in 1917, when the Wardroppers, including Sylvia, were also living in Russia. After about three years, Sylvia Simpson and James Tait succeeded in devising a satisfactory bioassay by measuring the effect of adrenal steroids on the urinary $^{24}Na/^{42}K$ ratios in adrenalectomized rats after the injection of trace amounts of the radioactive isotopes (9, 11). It was found to be important to avoid loading the rats with electrolytes, as would have been necessary with the use of the insensitive flame photometers then available, to measure the appropriate non-radioactive electrolytes. With the injection of trace amounts of electrolytes, all adrenal steroids tested acted unidirectionally and this simplified the interpretation of the results when assaying mixtures of steroids from adrenal extracts before extensive purification. Sylvia Simpson had already used quantitative methods, involving the analysis of variance, in bioassays and these were applied routinely in the $^{24}Na/^{42}K$ assay even though this involved the rather hectic use of mechanical calculators between assays. The excellent facilities in the Courtauld animal house meant that the rats used were bred in house in excellent conditions, and a narrow weight range of animals could be selected. The assays were conducted in conditions of constant temperature and humidity available in certain experimental rooms in the animal house, which was important for assays involving electrolyte metabolism. The development of this sensitive, specific and reliable assay was undoubtedly the key factor in the subsequent success of its application at the Middlesex, particularly with the initially surprising and potentially confusing results. Later, the Mayo Clinic group, led by H. L. Mason and V. R. Mattox (Mason *et al*. 1936; Mattox *et al*. 1953), applied a similar bioassay but measured the non-radioactive Na/K ratio with a sensitive flame photometer. This gave the same results for the activity of steroids between the Middlesex and Mayo Clinic groups (72), as was established by direct comparisons of the two types of assay at the Middlesex (21).

With the collaboration of Hilary Grundy, Simpson and Tait found that a commercially available ox adrenal extract (Eucortone, from Allen & Hanbury) showed high activity in the $^{24}Na/^{42}K$ assay (10), as had the amorphous fraction obtained by earlier workers in the adrenal field after the crystallization of the known adrenal steroids (Mason *et al*. 1936; Kuizenga 1944). A crucial technique in these studies was the use of ultraviolet light (254 nm) to detect the usual adrenal steroids on the paper chromatograms non-destructively (66). As it later emerged, this technique was developed and used independently by Ian Bush (Bush 1952) and the Upjohn group (Haines & Drake 1950). As in the earlier work on the amorphous fraction, this activity in Eucortone could not have been due to deoxycorticosterone, the model mineralocorticoid synthesized by Tadeus Reichstein (ForMemRS 1952), as it was present in negligible quantities (10) (Kuizenga 1944). However, the activity could theoretically have been due to a synergistic action between known adrenal steroids, as was suggested as a possibility for

the amorphous fraction in an influential review by Sayers (1950). The Middlesex team then fractionated the adrenal extract using the recently published Zaffaroni paper chromatographic system (propylene glycol/toluene) (Zaffaroni *et al*. 1950; Burton *et al*. 1951) (12, 14). At the time, the clinical groups of Conn and Albright had proposed a unitarian theory of adrenal secretion, with cortisol being both the natural glucocorticoid and mineralocorticoid (Fourman *et al*. 1950; Conn *et al*. 1951). This theory arose mainly because the therapeutic use of large amounts of cortisone (equivalent to the administration of similar quantities of cortisol) had caused marked effects on sodium and potassium metabolism. Therefore, when the assay of the Zaffaroni chromatogram (propylene glycol/toluene) of adrenal extract by the Middlesex team showed that nearly all the electrolyte activity ran at exactly the same speed as cortisone and it could not be separated from cortisone under the usual conditions (running for three days), this result might have seemed to provide a confirmation of the unitarian theory (10, 12, 14). However, experience with the electrolyte assay had shown that the amount of cortisone in the elution could not account for the activity (10, 11). It was an advantage in this situation that, in this bioassay, all known adrenal steroids acted unidirectionally to decrease the $^{24}Na/^{42}K$ urinary ratio. Eventually it was found possible to separate the electrolyte activity and cortisone in the Zaffaroni system by running the system for seven days (12, 14). In addition, in the very different paper chromatographic system devised by Bush (1952) (12, 14, 15) the active compound ran clearly between cortisone and cortisol with the solvent system benzene/aqueous methanol. This type of solvent system, used by Bush for paper chromatography (at raised temperatures), was also employed by Morris and co-workers for the partition column chromatography of steroids at room temperatures (Butt *et al*. 1949; Morris & Williams 1953). Another unique property of the active compound was that, after acetylation, the derivative chromatographed as a polyacetate, which was inactive in the $^{24}Na/^{42}K$ assay. However, after acid hydrolysis, some activity, presumably due to the free compound, could be regenerated by acid hydrolysis, enabling the chromatographic properties of the polyacetate to be established (15). These results proved that the activity in the adrenal extract (and probably also in the earlier amorphous fraction) was due to a single previously unknown highly biologically active compound. This was termed ‘electrocortin’ by the Middlesex team. During these studies it was tentatively concluded that electrocortin did not possess a Δ4-3-oxo structure because the relevant eluates of the paper chromatograms did not have an ultraviolet absorption peak at about 240 nm (12). However, later application of the sensitive and specific Bush soda fluorescence test (Bush 1952) indicated that it did have such a group (15). The previous negative result was due to the unexpectedly high biological activity (and therefore the very small amounts) of electrocortin present in the eluates. There was also a contaminating phenol (with an ultraviolet peak at 280 nm) that ran at about the same speed as electrocortin. This was later isolated and identified in Basle but was shown not to have any type of biological activity (22). As a result of this ‘error’ at the Middlesex, it was found that allotetrahydro metabolites of Δ4-3-oxo steroids, for example allotetrahydrocortisol or Reichstein’s compound C (allotetrahydrocortisol), were active in the $^{24}Na/^{42}K$ assay (half as active as the parent Δ4-3-oxo steroids) (72). The biological activity of allotetrahydrocortisol confirmed the earlier results of Reichstein with a mineralocorticoid assay (72). The importance of this is still not clear but it means that an active glucocorticoid, such as cortisol, can be converted to a mineralocorticoid, allotetrahydrocortisol, after metabolism in the liver (Hechter & Pincus 1954).

In his review, Sayers (1950) had also suggested that the electrolyte activity in the amorphous fraction could be a compound that was an artefact present in adrenal extract but not nec-

essarily secreted. The Middlesex team then collaborated with Bush, who was fortunately working at the time in the nearby MRC Laboratories in Mill Hill, to investigate this possibility. In addition to devising paper chromatographic methods for adrenal steroids, as described previously (Bush 1952), Bush had successfully made *in vivo* preparations of perfused adrenal glands for various animals based on the earlier work of Martha Vogt (FRS 1952) (Vogt 1943). Bush had analysed their secretion for specific adrenal steroids with his paper chromatographic methods (Bush 1952) and found a marked species variation in the ratio of cortisol to corticosterone (Bush 1953). However, although aware of the possible significance of active compounds in the amorphous fraction and not believing in the unitarian theory, he did not have a suitable bioassay to enable him to investigate this aspect. The Middlesex and Mill Hill teams then collaborated and in a short period of intensive work showed that electrocortin was secreted (13). It was found that the electrolyte-active compound in the secretions of monkey and dog adrenal glands behaved chromatographically in exactly the same way as electrocortin obtained from adrenal extract. In addition, this active compound in an extract of dog perfused blood showed the same unique properties as electrocortin after acetylation. Meanwhile, John Luetscher in the USA had shown mineralocorticoid activity in human urine (Deming & Luetscher 1950; Luetscher *et al*. 1954), which was revealed, after correspondence by the Middlesex team with John Luetscher, to be identical with electrocortin. Therefore, in 1952, it was proved definitively that electrocortin was secreted by the mammalian adrenal gland and was, by the usual definition in endocrinology, a new hormone.

The Middlesex team then prepared 1 mg of pure electrocortin by using column partition chromatography (15), devised for separating steroids in blood by Morris and co-workers (Butt *et al*. 1949; Morris & Williams 1953). This preparation showed the same infrared spectra (both as the free compound and as the acetyl derivative) as the crystalline material prepared later by Reichstein. The application of specific chemical micromethods in the Middlesex (including by Dr Kellie's group in the Courtauld) showed that electrocortin had both the Δ4-3-oxo and the α-ketol sidechain structure possessed by most other biologically active adrenal steroids (15). By using ^{14}C-carboxy-labelled acetic anhydride, it was established by the Middlesex team that electrocortin had a total of two acylable groups, one at position 21 and another at an unknown position (15). Infrared spectra indicated that this carboxyl group in the electrocortin diacetate was in proximity to the 21-carboxyl group in the side chain, such as at the 16 position, but it was not further characterized at this time (15). As a result of these preliminary findings, 16-hydroxydeoxycorticosterone was synthesized in several laboratories. It was found to be active as a mineralocorticoid but later 16-oxygenated steroids were found to cause salt excretion.

At this point it became clear that much larger quantities of electrocortin must be prepared in order to perform classical degradation studies to establish the structure rigorously. As a result mainly of the good offices of Sir Charles Dodds, Simpson and Tait then collaborated fully with Reichstein to achieve this goal. The Ciba team of A. Wettstein, R. Neher and O. Schindler in Basle was also involved, and Organon (Holland) was essential in supplying the large amounts of adrenal extract required. The collaboration is fully documented in the extensive correspondence between Reichstein and the Middlesex group (72). A summary of this was published (72) and the original letters are in the Wellcome Museum of the History of Medicine, London. This correspondence indicates that the use of the ^{24}Na/^{42}K bioassay in London was crucial in guiding the isolation work after, at a critical point, the failure of paper chromatographic methods of analysis in Basle (72). Eventually, 21 mg of crystalline electrocortin was obtained in Reichstein's laboratory and subsequently smaller amounts of crystalline

material in the Middlesex and in the Ciba laboratories in Basle (16, 18). The 21 mg obtained by Reichstein was sufficient for him to arrive at the correct unexpected structure, a remarkable achievement in those days (17, 19). The finding of Δ4-3-oxo and α-ketol groups in the structure of electrocortin by the Middlesex team, was confirmed by more rigorous methods. However, the extra acylable hydroxyl group was found to be at the 18 position in a hemiacetal structure with 11β-hydroxyl and 18-aldehyde groups (Fieser & Fieser 1959) (17, 19). Both these groups were concealed (for example in the infrared spectra) by the hemiacetal formation, which is probably formed nearly 100% in solution (Neher 1979) (17, 19). The structure of electrocortin was finally elucidated as 11β,21-dihydroxy-18-oxo-pregn-4-ene-3,20 dione and, with the agreement of Simpson and Tait, Reichstein renamed electrocortin aldosterone (17, 19). Only a few weeks after the successful efforts of Reichstein, the structure of aldosterone was confirmed by similar chemical studies by Mason and co-workers at the Mayo Clinic (Mason *et al*. 1936; Mattox *et al*. 1953) and later by Sarrett and co-workers (Harman *et al*. 1954; Ham *et al*. 1955). Finally, aldosterone was synthesized by Schmidlin and co-workers at the Ciba laboratories (Schmidlin *et al*. 1955). The rigorous elucidation of the unique structure of aldosterone was due almost entirely to Reichstein. The main contribution of Simpson and Tait in this later work on the structure was the previous establishment of the fully acetylated derivative as the diacetate, which was a crucial question at one point in discussions held in Basle between the collaborating groups (15, 72). This acetyl derivative was never crystallized satisfactorily, but the analysis with [^{14}C]acetic anhydride at the Middlesex supplied the crucial information accurately (15). This radioactive acetic anhydride method (preferably with ^{3}H-labelled acetic anhydride with [4-^{14}C]aldosterone as an indicator) was later used, for analysing small amounts of steroids in biologically material by Simpson and Tait and co-workers (20). It was later particularly successfully employed by Peterson and co-workers (Kliman & Peterson 1960), who used the commercial liquid scintillation counters then available in the USA to analyse the very small amounts of aldosterone in biological fluids. Nearly all the extensive international studies (for example at the Howard Florey Institute of Experimental Physiology and Medicine in Melbourne) on the mode of control of aldosterone used the radioactive acetic anhydride method to measure aldosterone.

After the elucidation of the structure of aldosterone, P. Ayres and P. Gould, at the Middlesex, in collaboration with Simpson and Tait, found that aldosterone was produced only in the zona glomerulosa of rat and beef adrenals and cortisol by the zona fasciculata-reticularis of beef glands. Corticosterone was produced by all regions of the adrenal cortex in both species (38). At about the same time, Giroud and co-workers (Giroud *et al*. 1956), in Montreal, found the same distribution of steroids produced in the rat adrenal. Later, the Middlesex team found a similar distribution of steroids produced by the human adrenal (39). These results confirmed conclusions from the more indirect earlier studies of H. W. Deane, R. O. Greep and others (Deane *et al*. 1948).

In biosynthetic studies, the Middlesex team, led by Simpson and Tait, and partly in collaboration with the team of Oscar Hechter at the Worcester Foundation for Experimental Biology (WFEB), Shrewsbury, MA, USA, found, by using radioactive steroids as substrates in adrenal incubations, that progesterone, deoxycorticosterone and corticosterone were major precursors of aldosterone (41, 69). The result with corticosterone was unexpected because Hechter in his previous classical studies of the biosynthesis of corticoids at the WFEB had concluded that ‘the adrenal enzymes regarded 11β hydroxylation as the trade mark of an end product’ (Hechter & Pincus 1954).

The metabolism of aldosterone was also studied extensively by the Middlesex team. [^{3}H]aldosterone, specifically labelled at the 16 position, was biosynthesized with, as substrate, tritiated [16-^{3}H]progesterone synthesized by W. H. Pearlman, who was then working in London (23, 40). Pearlman had also been interested in steroid dynamics (Pearlman *et al.* 1954), which influenced work at the Middlesex. The tritiated aldosterone had a high specific radioactivity and could be injected in trace amounts to study metabolism. It was found that this labelled aldosterone had a high volume of distribution and metabolic clearance rate as expected from its low-affinity binding to proteins such as transcortin in human blood and in contrast with the metabolism of cortisol (23–25, 27). A method of measuring aldosterone secretion rates was devised from the specific radioactivity of a urinary metabolite (23, 24) (the acid-labile metabolite of aldosterone itself discovered by J. Luetscher (Deming & Luetscher 1950; Luetscher *et al*. 1954)). This method, based on the earlier work of Pearlman, who used oestrogens labelled with non-radioactive deuterium to estimate oestrogen secretion rates (Pitt 2003), was subsequently used generally in the steroid field as a relatively non-invasive method to measure secretion rates of steroids in humans. The theoretical basis of the method was studied later by the Taits at the WFEB (28, 29).

Sylvia Simpson and James Tait married in 1957, but Sylvia continued to use her married name professionally. This meant a change in name from Simpson to Tait, which has caused some confusion to newcomers in the field. In 1958, as a result of interdepartmental political difficulties in the Middlesex and because they knew and admired scientists in their intended new workplace, the Taits decided to accept the invitation from Gregory Pincus to move to the WFEB in the USA. Pincus was best known for his work on the contraceptive pill (Pincus 1965). However, early preparations of the pill containing compounds with combined oestrogenic and progestational effects had increased the secretion of aldosterone, a potentially harmful side effect (Pincus 1965). At this point, the use of the ^{24}Na/^{42}K assay, which had done such noble service, was terminated in the tradition—expounded by Sir Henry Dale FRS—that the best use of a bioassay method was to eliminate itself.

At the WFEB, the Taits first continued their studies on aldosterone metabolism with [7-^{3}H]aldosterone and [4^{14}C]aldosterone synthesized there in collaboration with Marcel Gut, a chemist and former student of Reichstein's (25, 30–34). The properties of the acid-hydrolysable metabolite of aldosterone were investigated in collaboration with R. Underwood (29). These properties were explained by the structure, proposed by Underwood and confirmed by Maddox and Mason, as being the 18-glucuronide. With the collaboration of A. Brodie, A. Riondel, R. Horton and B. Little, the studies on the metabolism of aldosterone, both experimental and theoretical, were extended to other steroids, such as progesterone, androstenedione and testosterone. The method of continuous infusion of trace amounts of labelled steroids to estimate their metabolic clearance and interconversion rates was applied (27, 32). In addition, labelled reagent methods, using [^{3}H]acetic anhydride, [^{14}C]acetic anhydride and [^{35}S]thiosemicarbazide, were employed to measure the peripheral plasma concentrations of the steroids (31, 35, 36). These measurements allowed the calculation of plasma production rates and the contribution of precursors to these production rates. These studies concluded that when there was peripheral interconversion of steroids, as for androstenedione and testosterone, the measurement of plasma production was easier to interpret than values obtained from analysing urinary metabolites. It was also concluded that androstenedione could be a prehormone, which was important to young women in determining local concentrations of testosterone.

At the WFEB, the Taits also advised on the study of the effects of the contraceptive pill and its constituent compounds on adrenocortical metabolism, particularly the secretion and metabolism of aldosterone and cortisol (Layne *et al*. 1962) (26). It was found that the secretion of aldosterone was increased as a result of the effects of the progestational component and the plasma binding of adrenal steroids by the oestrogenic element (Layne *et al*. 1962). These effects could readily be normalized by reducing the dose of the components of the pill without significantly affecting its contraceptive efficiency.

An indirect non-invasive method employing the administration of oral [^{14}C]aldosterone and intravenous [^{3}H]aldosterone followed by the measurement of the isotopic ratio of urinary aldosterone predicted the nearly 100% hepatic extraction of aldosterone in normal subjects. In collaboration with J. Bougas and B. Little, the Taits measured the hepatic extraction and metabolic clearance rate of aldosterone directly in patients with congestive heart failure (34). It was found that the hepatic extraction of aldosterone was lowered in congestive heart failure, depending on the severity of the condition. This was also found by Luetscher and co-workers at about the same time (Cheville *et al*. 1966). This finding may be relevant to the escape of lowered concentrations of aldosterone in patients with congestive heart failure after treatment with angiotensin-converting enzyme (ACE) inhibitors and the success of therapy with aldosterone inhibitors such as spironolactone (Sayers 1950; Selye & Horava 1953; Luetscher *et al*. 1954; Schmidlin *et al*. 1955).

After about two years at the WFEB, the Taits studied the biosynthesis of aldosterone *in vivo* in sheep at the Physiology Department, University of Melbourne, Australia, headed by Professor D. Wright. These studies were performed with a research group in the department, led by Derek Denton (FRS 1999), who had successfully transplanted the left adrenal gland to the neck of Merino sheep. The right adrenal gland had been removed at an earlier operation. Studies of the group indicated that these glands functioned normally. The advantage of these transplanted glands was that they were readily accessible so that the biosynthesis of aldosterone could be studied under various *in vivo* conditions, such as in different states of sodium balance, without stressing the animal. It was found that in the lower range of aldosterone secretion with moderate salt deficiency, the rate of conversion of corticosterone to aldosterone increased at about the same rate as the increase in aldosterone output (42, 43). However, with severe sodium deficiency, the conversion rate decreased markedly with increasing aldosterone output (42, 43). Later the group at the Howard Florey Institute suggested that, although after moderate salt depletion corticosterone was probably an important precursor of aldosterone, after severe salt depletion an alternative pathway, such as that involving 18-hydroxycorticosterone, might be activated (Boon *et al*. 1996). This stay in Australia was an invaluable if exhausting experience, both at work and play, that the Taits never forgot. It made for fruitful collaboration and friendship with members of the group, particularly Derek (Dick) Denton, John Coghlan and Marelyn Winter-Coghlan, for the rest of their lives. Unfortunately, both the leading animal surgeons involved, Douglas Wright and Jim Goding, died prematurely. Shortly before Wright's death, the Taits were privileged to give the Wright Lecture and Seminars in the University of Melbourne.

Although Sylvia Tait played a full role in the experimental work, the studies of steroid dynamics at the WFEB, particularly the theoretical aspects, were mainly the concern of James Tait. However, eventually the Taits also decided to study isolated adrenal cells, particularly zona glomerulosa (ZG) cells from rat glands, with a view to helping to elucidate the molecular basis for the control of aldosterone production. The WFEB was an appropriate place to do

such work: Oscar Hechter and co-workers had pioneered the use of isolated dispersed cells generally. In the studies on isolated adrenal cells, although James Tait had a significant role, for example in planning experiments, this line of research became the major interest of Sylvia Tait, and she supervised this experimental work. Ray Haning, a Steroid Training Course Fellow at the WFEB, had a vital role in these pioneering studies on ZG cells. Eventually, a reliable system using collagenase was developed to produced isolated ZG cells from capsular strippings of rat adrenals. The aldosterone output of these cells *in vitro* responded reasonably to the known *in vivo* stimuli, such as K^+, angiotensin II and corticotropin (ACTH), and also to serotonin (44). Unfortunately, the usual preparation of ZG cells obtained from rat adrenal capsular strippings contained 5% of contaminating zona fasciculata-reticularis (ZF-ZR) cells. This complicated the interpretation of the results of experiments with stimuli such as ACTH, which increased the output of both ZG and ZF-ZR cells. This is because, in the usual *in vitro* arrangement, the corticosterone produced by ZF-ZR cells can act as a precursor for the production of aldosterone by the ZG cells. Presumably this does not occur *in vivo* with the usual routes of circulation of blood through the adrenal gland. Using cells from capsular strippings, ACTH gave the greatest response of aldosterone to any stimuli tested, but this was probably largely due to the contaminating ZF-ZR cells. However, the characteristics of the response of the capsular cells to K^+, with a maximum effect at about 8 mM K^+, was established and this was not affected by the contaminating ZF-ZR cells, whose steroid response did not respond to increases in K^+ concentration (44).

In 1970 the Taits returned to the Middlesex Hospital Medical School. The main reason was that there were family problems in England that required their attention. Gregory Pincus, to whom they had obligations, had died and a new suitable Director of the WFEB, Mahlon Hoagland, had been appointed. James Tait returned to the Middlesex as Head of the Department of Physics as Applied to Medicine, and a Biophysical Endocrinology Unit, led by the Taits and mainly supported by the UK MRC, was established within the department. The work in the Biophysical Endocrinology Unit concentrated on the studies with the isolated adrenal cells, which had been Sylvia's main interest (50, 54). However, one of the first tasks of the new team was to use the resources of the Physics Department to purify the rat adrenal cells by the method of unit gravity sedimentation. The cells were detected by Coulter counting with pulse height analysis. Vital to this work was the collaboration of Peter Gould, an electron microscopist in the Biology Department, who had participated in the early adrenal morphological work of the Taits at the Middlesex. A biochemist in the Biophysical Unit, Janet Bell, also collaborated from the start of the work on isolated cells and later, P. Hyatt, another biochemist with interests in morphology, also joined the team. Using unit gravity sedimentation, ZG cells from rat capsular strippings were purified satisfactorily (45). There was a large loss of cells due to clumping, which was difficult to prevent. However, the remaining cells were in a better state than in the original preparation, according to electron microscopy. With the purified ZG cells it was shown that ZG cells respond to ACTH as expected from *in vivo* results. The maximum response to ACTH was now similar to that of stimuli, such as serotonin and K^+, that increased the aldosterone output of ZG cells only. At high concentrations, the preparation of angiotensin II used initially increased the aldosterone output of unpurified capsular cells to about the same extent as ACTH. However, mainly through the work of D. Schulster, who was then at the MRC laboratories at Hampstead, this was shown to be due to contamination of the angiotensin II, probably by ACTH, in this preparation. A pure angiotensin II preparation stimulated the aldosterone output of capsular and pure ZG cells to

the same extent as the other stimuli and did not stimulate rat ZF-ZR adrenal cells, as was also found by Kevin Catt (57). By then Catt and co-workers also were studying isolated adrenal cells after collagenase treatment in the NIH laboratories in Bethesda, MD, USA. Therefore, to summarize, it was shown at the Middlesex that the aldosterone output of purified ZG cells from rat adrenal cells responded to K^+, ACTH, angiotensin and serotonin at reasonable concentrations of the stimuli and that the maximum responses to all these stimuli were similar. This corresponded to *in vivo* results when the comparison could be made (45–49, 51, 56).

As a less demanding method compared with unit gravity sedimentation, John McDougall (from the Howard Florey Institute and holder of an overseas fellowship financially supported by the Royal Society) and B. Williams had found that ZG and ZF-ZR cells could be separated by column filtration (53). These workers also found that the Ca^{2+} efflux of superfused ZG cells held on a column was increased by angiotensins II and III but not by K^+, serotonin and cyclic AMP (58).

As a rather fortuitous bonus to the work to obtain pure ZG cells, it was found readily possible in the same sedimentation run to obtain pure ZR cells from the capsular cells (52, 55, 56). In addition, using unit gravity sedimentation, these ZR cells could be obtained in greater quantities from preparations of rat decapsulated adrenals, which consisted nearly entirely of ZF-ZR cells with only a few contaminating ZG cells. These ZF-ZR cells produced mainly corticosterone, the major glucocorticoid in the rat, but no aldosterone. At the time there were two main theories about the function of the ZR cells. The cell migration theory stated that the region of the adrenal containing ZR cells was one of low mitotic activity, where the adrenal cells migrated to die. In contrast, the functional theory proposed that the ZR cells secreted steroids with a different function from that of the ZF cells; for example the ZR cells produced the adrenal androgens preferentially (Zaffaroni *et al*. 1950). It was found with the preparations of pure cells obtained at the Middlesex that there was a deficiency in 11β hydroxylating activity by ZR compared with ZF cells. This would lead to the preferential production of 11-deoxy steroids, including adrenal androgens such as androstenedione, in ZR cells with a diminished production of corticosterone. Additionally, the response of corticosterone (and cyclic AMP) output to ACTH was lower in the ZR cells. These observations were the first direct demonstration that the functional theory for ZR cells was probably correct, although it did not exclude the cell migration theory from applying simultaneously. These results were extended to studies with guinea pig adrenals, which had the advantage that the ZF-ZR cells produced cortisol as for human adrenals. This later work on ZR cells, which confirmed the earlier work at the Middlesex on rat adrenal cells, became the special interest of P. Hyatt, who had joined the Unit.

As regards studies on the molecular basis of the mode of action of ZG stimuli, the Middlesex team, with the collaboration of B. Brown and R. Ekins in the Nuclear Medicine Department of Middlesex Hospital, first studied the role of cyclic AMP. It was found that ACTH and serotonin definitely stimulated cyclic AMP in the ZG cells. As in studies with ZF-ZR adrenal tissue, there was some dichotomy in the steroid and cyclic AMP responses but this could be explained theoretically. When pure angiotensin II was used as an aldosterone stimulus, cyclic AMP was not increased in rat ZG cells, as was first established by the group of K. Catt in Bethesda. Actually, both groups found that the cyclic AMP output was decreased slightly but significantly by pure angiotensin II. However, the effect of increases in K^+ concentration on the cyclic AMP output of rat ZG cells remained controversial. The Middlesex team found that increases in K^+ concentration did increase cyclic AMP significantly but the

corresponding results of the Catt's group were negative. Eventually, it was accepted generally in the field that K^+ did increase cyclic AMP. The negative results of the Bethesda group were probably due to the use of phosphodiesterase inhibitors, which had unexpected effects.

Also in the Tait laboratories, G. St J. Whitley and co-workers found that, in ZG cells, the angiotensins markedly increased the incorporation of [^{32}P]phosphate into phospholipids, such as phosphatidylinositol and phosphatidic acid, and also [^{3}H]inositol into inositol monophosphate, bisphosphate and trisphosphate (59, 60), indicating an effect on phospholipase C activity. ACTH, K^+ or serotonin were nearly equally effective as the angiotensins in stimulating steroidogenesis in ZG cells but had no such specific effects on the production of inositol phosphates. ACTH in ZF-ZR cells also had little specific effect on phospholipid metabolism. However, the angiotensins had a marked effect of incorporation of labelled inositol into the inositol phosphates in ZF-ZR cells, although the pure peptides did not stimulate steroidogenesis. This suggested that there was a lack of receptors for the inositol phosphates in ZF-ZR cells for these messengers to effect the stimulation of steroidogenesis with the angiotensins. Of all the stimuli of steroidogenesis in adrenal cells, only the angiotensins in ZG cells used inositol phosphates as effective messengers. α-Melanocyte-stimulating hormone (α-MSH) preferentially increased the steroidogenesis in ZG cells. However, as with ACTH, there was concomitant stimulation of cyclic AMP with α-MSH but not of phospholipase C in either ZG or ZF-ZR cells (61).

At this point, in 1982, both the Taits decided to retire from the Middlesex. A major factor in this decision was that the atmosphere in London University, with the constant pressures to eliminate at least one Medical School, was not conducive to conducting good research. Apart from the waste of time in the relevant political discussions, this made it difficult to retain research staff of high quality; potential collaborating colleagues such as Professor T. Powell, Physiology Department, University of Oxford, who had succeeded in producing a viable preparation of isolated heart cells, left the department for an appointment in Oxford University. After the Taits retired to their house in East Boldre in the New Forest, Hampshire, P. Hyatt continued studies on ZG cells in the Physics Department, particularly the role of Ca^{2+} in the action of ZG stimuli, such as increased K^+ concentration (62). The Taits, who had good modern facilities, including computing, in East Boldre, were in close contact with the work and helped to interpret the data and design of experiments. It was concluded that changes in internal cellular Ca^{2+} concentration were important in the action of increased K^+ to stimulate aldosterone production through cyclic AMP and other mechanisms (62).

In the later 12 years of studies at the Middlesex on the molecular basis of the mode of action of stimuli of ZG cells, the Taits and co-workers showed the following. First, ACTH, α-MSH and serotonin stimulate steroidogenesis to different extents in ZG and in ZF cells. Serotonin stimulates the steroidogenesis of ZG cells only. ACTH is the most active stimulator of ZF compared with ZG cells. α-MSH is more of a ZG stimulator than ACTH is, but is as specific as serotonin. These different activities in stimulating steroidogenesis in the ZG, ZF and ZR cells may be correlated with binding properties of the stimuli to these cells, although this has not yet been established. All these three stimuli use cyclic AMP as a messenger in ZG, ZF and ZR cells. Second, AII, AIII and angiotensin analogues stimulate phospholipase C in ZG cells by means of changes in the levels of intracellular Ca^{2+} and the inositol phosphates. Phospholipase C is also increased in ZF cells by the angiotensins. However, only in ZG cells are the inositol phosphates used as messengers to increase steroidogenesis. Third, K^+ acts to increase steroidogenesis in ZG cells through changes in intracellular Ca^{2+}, which probably

acts as the primary messenger. These changes in Ca^{2+} may also increase cyclic AMP levels but the relative importance of this is not known.

In 'retirement' in East Boldre, the Taits continued to publish scientific papers even after their research unit in the Middlesex closed. Using computer simulation (with two Apple IIe personal computers operating in parallel), the effect of the binding of steroids to albumin in circulating blood on the hepatic extraction of steroids was modelled (37). It was concluded that an active process must be involved to explain the high hepatic extraction of steroids strongly bound to albumin (37). The Taits also published scientific reviews, such as on the effects of K^+ on cyclic AMP in ZG cells (73), and a theory to explain the hypertension of NIDDM patients (70). They also wrote historical scientific reviews on the development of the concept of metabolic clearance rate (71) and continued to write, mainly on request, accounts of the discovery and identification of electrocortin (aldosterone) (63–65, 67, 68, 72, 74). The last joint publication of the Taits was written in collaboration with John Coghlan for the meeting in London celebrating the 50th anniversary of the discovery and identification of aldosterone (73). This meeting was held on 24 April 2003 at the Royal Society building in Carlton House Terrace, London. Unfortunately, Sylvia Tait died on 28 February 2003 and James Tait was in the Royal Bournemouth Hospital being treated surgically for the long-term effects of diabetes. Therefore, neither of the Taits could attend the London meeting and their paper was given by Coghlan. There was much interest in this meeting clinically because it had been established, mainly in Brisbane, Australia, that primary aldosteronism was a much more common condition than had been supposed (occurring in 10–15% of all hypertensive subjects) (Stowasser & Gordon 2003). Conn first described the condition (Conn 1955) and proposed its relatively high frequency and occurrence even with nearly normal blood K^+ concentrations. The more recent confirmation of the relatively high rate of occurrence of the condition was due mainly to the use of the ratio of renin to aldosterone concentration in peripheral blood as a diagnostic tool (Stowasser & Gordon 2003). Surgical removal of the relevant tumour (usually an adenoma without metastases) has made primary aldosteronism the most treatable form of hypertension (Young *et al*. 1994). In addition, it had been found that treatment of patients with congestive heart failure (using ACE inhibitors) with an anti-aldosterone compound, such as spironolactone (or eplerenone), markedly reduced mortality and hospital admissions (by about 30% for spironolactone) (Pitt et al. 1999, 2003; Jessop 2003; Pitt 2003). This is probably because aldosterone can produce cardiac dysfunction, such as that due to fibrosis in the heart (Young *et al*. 1994). Ironically, after suffering from very painful leg ulcers for about two years, Sylvia Tait developed a heart condition which seems to have been possibly suitable for treatment with spironolactone. However, the treatment actually used proved to be increasingly ineffective and she died of renal and heart failure in Lymington Hospital, Hampshire, just before the 50th anniversary meeting. James Tait, temporarily allowed out of the Royal Bournemouth Hospital, visited her bedside on her last day. After a service at Bournemouth Crematorium, Sylvia's ashes were placed in Lymington Cemetery in a small plot with a simple marker, where those of James Tait will also be placed. When she died, she was the senior woman Fellow of the Royal Society living in Britain. The senior woman Fellow at the time, Martha Vogt, who was then living in San Diego, had also been in the adrenal field, as described above (Vogt 1943).

Those who collaborated with Sylvia have warm and enduring memories of her enthusiasm, her direct and outspoken objective analysis of the data, and the pleasures of debate with

her. She disciplined herself with regard to any emotional attachment of her own hypotheses; accordingly, discussion often far into the night with Sylvia and James was relaxed though animated. She tended often to defer to 'Jimmy' on matters of everyday life and plans, but on issues of science it was an egalitarian partnership. In the early days there was division of responsibility with their work, with Sylvia taking major responsibility for the biological assay work and running the animal facility. She had an excellent team of technicians there, led by the Graves brothers, and she had a knack of inspiring loyalty in them and others. Her training as a zoologist was crucial in the organization of the animal experiments, particularly in as far as it involved adrenalectomized animals. The rats used were bred on site, and this enabled the careful selection of animals that contributed to the success of the critical bioassay. James Tait developed physicochemical methods for simultaneously measuring radioactive sodium and potassium as used in the bioassay, for the ultraviolet location of steroids on paper, and also the labelled acetic anhydride work that determined the number of acylable groups in the molecule. These data were noted by Reichstein as vital to the structural work on aldosterone. They were both equivalently involved in the theoretical interpretation of data, as was the case with the early investigation involving the discovery of aldosterone. This pattern of shared responsibility was evident when they worked with the Howard Florey group in Australia.

As a husband and wife team at the forefront of scientific discovery with great implication in general biology and medicine they were exceptional. Sylvia has left a legacy in the annals of scientific discovery, and is and will be honoured for it.

Positions held

1941–44 Department of Anatomy, Oxford; Assistant to Professor J. Z. Young
1944–55 Biological Assistant, Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, UK
1955–58 External Scientific Staff, Medical Research Council, UK
1958–70 Senior Scientist, Worcester Foundation for Experimental Biology, Shrewsbury, MA, USA
1970–85 Middlesex Hospital Medical School, London, UK, Department of Medical Physics (Research Associate 1970–82; Joint Head (with J. F. Tait) of Biophysical Endocrinology Unit 1970–85)
1985– Member at Large, Howard Florey Institute of Experimental Physiology and Medicine, Melbourne, Australia
1996– Honorary Member, Department of Molecular Endocrinology, University College London

Appointments

1981–83 Royal Society Sectional Committee 9
1985–89 Royal Society Library Committee

Awards

1959 Elected Fellow of the Royal Society of London
1976 Tadeus Reichstein Award of the International Endocrine Society
1977 Gregory Pincus Memorial Medal
Ciba Award, Council for High Blood Pressure
1979 Dale Medal, Society for Endocrinology
Honorary DSc, Hull University
1989 The R. Douglas Wright Lecture and Medallion

Membership of societies

Society for Endocrinology, UK
Endocrine Society, USA
American Association for the Advancement of Science
Royal Society of London

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The frontispiece photograph was taken in 1977 by Godfrey Argent, and is reproduced with permission.

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