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The discovery, isolation and identification of aldosterone: reflections on emerging regulation and function

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Abstract

This paper has a focus on the early history of aldosterone. The Taits take us on a chronological trawl through the history in which they had a first hand role and made a major contribution—their bioassay was in many ways the key. The gifted Swiss chemists made a critical contribution to the scale and isolation of larger amounts. This was international collaboration at its best. Developing technologies were utilised as crucial cutting edge applications in the advancing front, technology transfer before the word was invented.

Measurement of aldosterone and angiotensin were crucial advances to the understanding of the regulation of the hormone. In the period 1960–2003, some 30,000 papers mentioned aldosterone as a keyword, even so advances on a larger scale were slow. I have indicated some of my own work with the Howard Florey team using the adrenal autotransplant in the conscious sheep. Recently, the understanding of the role of induced proteins, the flow on from the RALES trial and the development of eplerenone has revitalised the aldosterone field.
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1. Introduction

As the abstract deadline approached, changes in the opening session became mandatory. One injunction from our major sponsor was that the meeting should not be a retrospective, but should focus on cutting edge research. Thus, the original program was going to have only one historical session. The Taits, both Sylvia and Jim, were on the program to address the discovery, isolation and identification of aldosterone, until about 1960, and I was slated to do from 1960 to the present, my own thoughts on the emerging understanding of regulation and function. Sylvia and Jim with their characteristic efficiency have had their paper ready for more than a year. Deteriorating health issues made their participation increasingly improbable, and you all know that Sylvia is deceased. After the most trying 12–18 months for her, 28th February saw her out of this life.

Jim remains severely restricted from complications arising from diabetes. In the event of their unavailability, it was discussed that I combined their paper with my own, I agreed to do so, recognising that this is very unusual, but I do so in the initial spirit in which the meeting was conceived.

Most of the pre-1960 comments are in their own words

We have previously presented several accounts of the history of aldosterone (Simpson and Tait, 1955; Tait and Tait, 1979, 1988, 1998b). We had resolved not to repeat such a review but the present occasion is obviously quite special. We have tried to add some comments from present day hindsight. The aldosterone field is now vast and a balanced review cannot be presented here. Therefore, apart from an account of the early history, we shall also unashamedly put forward some questions arising from past and present personal hobbyhorses.

as well as my own (J.P. Coghlan).

2. Early work on adrenal extracts

The first serious attempts to isolate the compound responsible for maintaining the life of adrenalectomised animals

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started round about 1935 following the preparation of biologically active extracts free of contaminating compounds from the adrenal medulla. The groups involved were principally those of: (1) Wintersteiner (Wintersteiner et al., 1934), mainly carried out at Columbia University, who, however, retired from the experimental field after a few years; (2) Kendall (Mason et al., 1936) at the Mayo Clinic isolated Cortin in crystalline form as early as 1934; and (3) Reichstein (Reichstein, 1936) and his faithful co-worker, von Euw, in Basle. Several other groups were involved in the 1930s, such as those of Hartman and Sport (1940) and Grollman (1937). Kuizenga and Cartland (1939) made notable contributions and a switch to the earlier extraction methods used

by them was one of the factors vital to the eventual success of Reichstein in isolating relatively large amounts of aldosterone in 1953 (Kuizenga, 1944; Simpson et al., 1953a,b; Tait and Tait, 1998b). Remarkably, Mason and Reichstein were still involved in the race for the isolation and identification of aldosterone in 1953 (Fig. 1).

In the late 1930s, it was realised that the life maintenance tests for adrenalectomised animals under low stress conditions favoured mineralocorticoids, such as deoxycorticosterone, but similar tests with stressed animals, e.g. under cold conditions, favoured glucocorticoids, such as cortisol (Swingle and Remington, 1944; Gaunt and Eversole, 1949, Table 1). As regards the assays used by the chemists, the

(a) From left to right: Prof. Reichstein, Sylvia Simpson, Dr. Tait, Prof. Wettstein, Dr. Neher and Joseph von Euw.
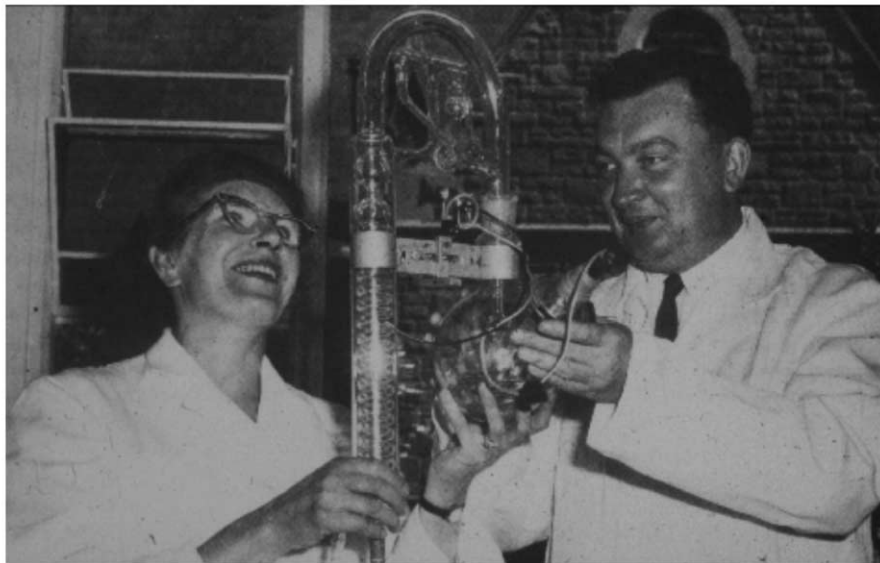
Fig. 1. (a) The full collaborative team with T. Reichstein (and the Ciba group, including Drs. A. Wettstein, R. Neher and A. Schmidlin). The Taits supplied them with all their information on the properties of electrocortin. Reichstein and von Euw then started the purification of the extract (by the Cartland and Kuizenga procedure) from 500 kg of beef adrenals. (b) Sylvia and Jim Tait "in their prime" February, 1962 in John Coghlan's laboratory at the Howard Florey Institute of Experimental Physiology and Medicine at the University of Melbourne. On study leave from the Worcester Foundation.

Table 1
Mineralocorticoid and glucocorticoid activity of corticosterone (B), cortisone (E), and deoxycorticosterone (DOC) (from Grundy et al., 1952a,b)

Bioassay (adrenalectomized rats)	B	E	DOC	E/DOC
Glucocorticoid assays				
Ingle (muscle fatigue)	100	210	4	53
Kuizenga–Venning (liver glycogen deposition)	100	192	–	–
Selye–Schenker (survival in cold)	100	1160	32	38
Mineralocortocoid assays				
Simpson–Tait (urinary Na^{24}/K^{42})	100	41	700	0.59
Everse de Fremery (muscle response)	100	44	1250	0.35
Cartland Kuizenga (survival low stress)	100	26	590	0.44

Ingle work test (Ingle, 1940), used by Kendall's group, measured chronic muscle fatigue and favoured glucocorticoids. However, the Everse and de Fremery assay (Everse and de Fremery, 1932), used by Reichstein, measured the immediate response of muscle and favoured mineralocorticoids. Nevertheless, this division of corticosteroids into these classifications was arbitrary and gluocorticoids exhibited some mineralocorticoid activity and vice versa. In any case, as a result of the application of these tests, many corticosteroids, both 'glucocorticoids' and 'mineralocorticoids', were crystallised and identified, particularly by both Kendall and Mason and Reichstein before 1940 (Swingle and Remington, 1944).

3. Amorphous fraction

It was realised by nearly all the early investigators in the field that the amorphous fraction remaining after the crystallisation of these steroids, contained considerable mineralocorticoid activity. This was not due to any known steroid, including deoxycorticosterone, a major candidate at the time (Kuizenga, 1944). From their biological activity and properties (Mason, 1939), it is clear that all these preparations containing substantial amounts of aldosterone with the best probably containing nearly 20% aldosterone by weight (Kuizenga and Cartland, 1939; Kuizenga, 1944; Mason et al., 1936; Wintersteiner and Pfiffner, 1936). If that was the case, then even the least efficient of the mild methods of purification available in recent times would have easily yielded pure crystalline material from such starting material.

Crystalline aldosterone was actually not obtained until 1953, about 18 years after the serious start of the search for this type of active compound in adrenal extracts. This was partially because the Second World War intervened and although there was still research activity in the adrenal field; this was mainly directed toward the possible use of glucocorticoids to reduce pilot fatigue. After the later post war, award of the Nobel Prize to Hench, Reichstein and Kendall mainly for work on glucocorticoids, there was then, it seems, a certain loss of faith in the significance of the amorphous fraction, except by the Mayo Clinic team.

4. Unitarian theory

The use of large amounts of steroids, such as cortisone, in clinical applications had made investigators realise that these glucocorticoids had effects on electrolyte metabolism, although these were regarded as undesirable side effects. It was also being realised from the content of adrenal venous blood in animals, as found by Vogt, Bush and Nelson (Vogt, 1943; Bush, 1953a,b; Nelson et al., 1950), and from the quantity of appropriate urinary metabolites, that cortisol was secreted in amounts similar to those used in therapy.

Therefore, for all these reasons, influential groups, such as those of Albright and Bartter (Fourman et al., 1950) and Conn et al. (1951) proposed that cortisol was the single adrenal hormone, which could control both carbohydrate and electrolyte metabolism. Even Bush (1985), who had paper chromatographed extracts originally prepared my Wintersteiner, stated that

I had almost convinced myself to join the Unitarians.

We should emphasise that the Unitarian views of all these workers were reasonably based on the evidence available at the time. On the other hand, the acceptance of the existence of aldosterone was certainly not a smooth development from clinical findings, as has been implied in a film of the history of the subject.

Actually, we now know, as emphasised by Ulick (1979, 1996), that cortisol can be a ubiquitous single adrenal hormone when there is excessive systemic or local production of cortisol, as in Cushing's or the AME syndrome. Even in normal subjects, cortisol contributes appreciably to the total mineralocorticoid activity, as can be seen in Table 1 (bottom figure). The mineralocorticoid activity was measured by our Na^{24}/K^{42} test and the glucocorticoid activity by the liver glycogen test (Fig. 2).

However, there is an important difference in the physiological role of aldosterone and cortisol. The secretion of aldosterone is controlled by such factors as the intake of electrolytes, so leading usually to a stable control system as the level of aldosterone in turn appropriately affects the excretion of these electrolytes. Cortisol is obviously part of a different, mainly ACTH based, control system. Although, Ulick (1996) and Beck et al. (1962) pointed out that cortisol production might increase in aldosterone deficiency and in normal subjects on a low salt intake, the physiological significance of this observation is not yet clear. In physiological situations, it presently seems that the aldosterone is the dominant hormone as regarding to the effects on electrolyte metabolism. However, this was certainly not the view of most people in the field around about 1950 at least as regarding to the role of an active compound in the amorphous fraction.

Apart from the views of Albright and Conn from clinical studies, as previously mentioned, there was a widely accepted opinion that the amorphous fraction could be due to several compounds acting synergistically. There was, in
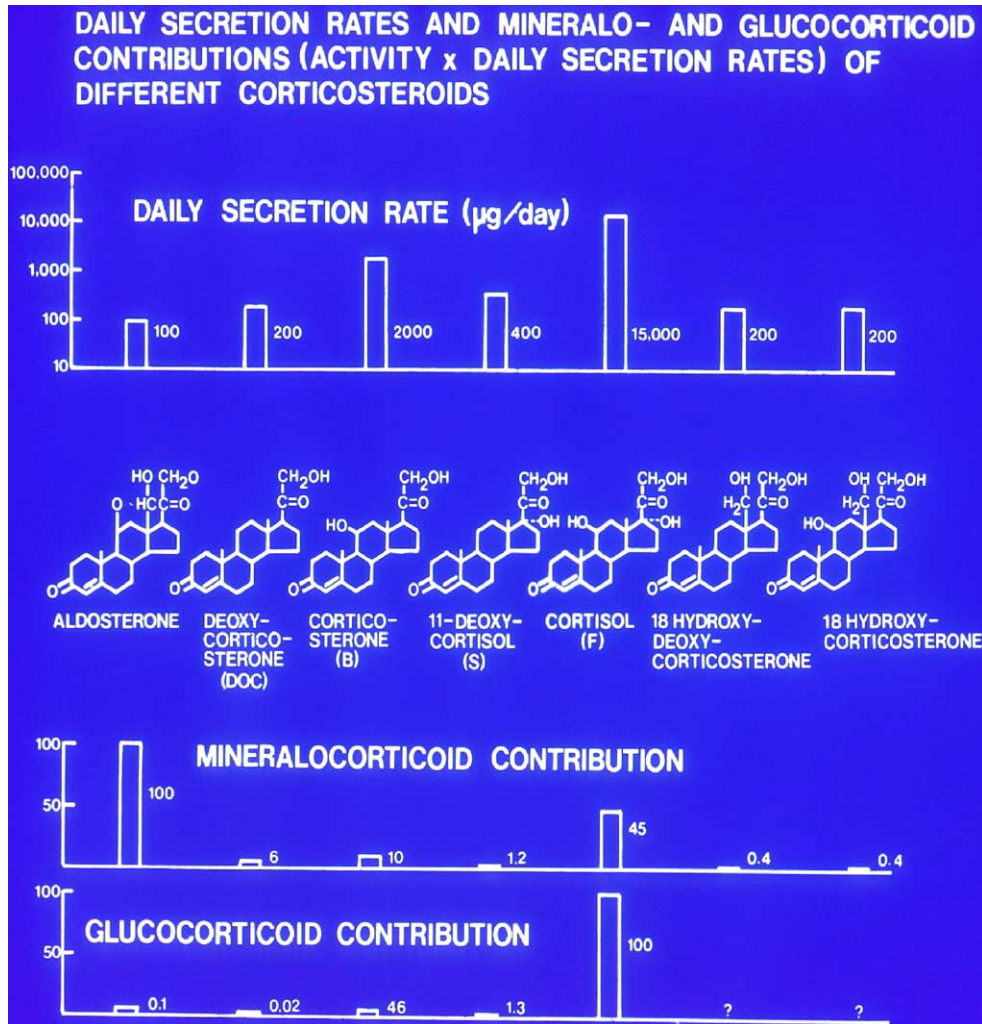
Fig. 2. Total mineralocorticoid and glucocorticoid activities of adrenocortical secretions (from Tait and Tait, 1979).

any case, no evidence for the actual secretion of a single active compound (Sayers, 1950). Also, the efforts of the very skilled chemical groups in the field, such as those of Kendall and Reichstein, had failed to isolate an appropriate active compound. It now seems that the relevant failure of the steroid chemists was probably due to an unfortunate accidental situation. The Kendall group used very mild methods of purification but the Ingle work test (Ingle, 1940), which they employed, was not sensitive to mineralocorticoids, such as aldosterone. On the other hand, Reichstein used the Everse and de Fremery assay (Everse and de Fremery, 1932), in which aldosterone is strongly active. Unfortunately, Reichstein also used alumina columns for isolating steroids and, employing this usually highly efficient technique, it was necessary first to acetylate steroids with an α-ketol side chain in order to prevent their destruction. At the time, all the known 21 acetyl derivatives of steroids were found to be nearly as biologically active as the corresponding free compound so this was not usually a problem. Unfortunately, under the same conditions of

acetylation, the 18,21-diacetate of aldosterone was formed and this was relatively inactive in the usual bioassays. This entirely unexpected phenomenon, probably markedly delayed the isolation of the hormone by Reichstein.

5. Direct elelectrolyte assays

In the late 1940s, various groups, all of them must still have believed in the existence of an active mineralocorticoid were devising methods that directly measured the action of steroid hormones on electrolyte metabolism (Dorfman et al., 1947; Spencer, 1950; Deming and Luetscher, 1950; Simpson and Tait, 1952; Singer and Venning, 1953). All these methods had greater convenience and sensitivity than the traditional bioassay methods, such as the Everse and de Fremery test. Particularly, worthy noting was the successful early method of Deming and Luetscher. Following a publication by Dorfman et al. (1947) pointed out to us by a clinician at the Middlesex Hospital, B. Lewis, we devised a bioassay

that measured the effects of steroids on the ratio of urinary radioactive sodium and potassium output of adrenalectomised rats after injection of only tracer amounts of the radioactive isotopes. With an injection of such small amounts of electrolytes, all steroids tested acted unidirectionally and this simplified interpretations of the results of the effects of biological extracts. Most direct microassays were applied to human urine but we, with Hilary Grundy, examined a commercial beef adrenal extract with the electrolyte ratio assay.

6. Applications of the Na^{24}/K^{42} assay

This assay immediately showed that Allen and Hanbury's adrenal extract was very active and this could not be due simply to the content of deoxycorticosterone or any other known steroid (Tait et al., 1952; Grundy et al., 1952a,b). However, this was no real advance on the conclusions of the earlier workers on the high activity of adrenal extract and could have been explained by synergistic actions between the known steroids (Kuizenga, 1944; Sayers, 1950). The extract was, therefore, fractionated by paper chromatography by the Zaffaroni method which used propylene glycol-toluene (Burton et al., 1951; Fig. 3).

Much to our surprise, and ironically, considering the prevailing Unitarian ideas of the time, the compound responsible for the biological activity ran at exactly the same speed as cortisone However, because of its low potency in the Na^{42}/K^{42} assay, cortisone could not directly account for the activity (Tait et al., 1952).

More directly, overrunning the Zaffaroni system, for 7 days, resulted in the complete separation of the active compound from cortisone (Grundy et al., 1952a,b; Fig. 4).

Later, the use of the Bush B5 paper chromatographic system (aqueous methanol:benzene) resulted in the rapid separation of the active compound from cortisone (and cortisol) (Simpson and Tait, 1953; Fig. 5). These chromatographic results definitely eliminated the possibility that the biological activity could be due to synergism between known steroids and strongly indicated that it was due to a single compound (then termed electrocortin).

It still remained to be demonstrated that electrocortin was secreted by mammalian adrenal glands. Fortunately, nearby in London, I.E. Bush was working at the MRC Laboratories, Mill Hill, where he had made preparations of perfused mammalian adrenal glands (similar to those devised by Vogt (1943)). He had found that there was a marked species difference in the secretion of adrenal steroids, particularly in the ratio of secreted cortisol to corticosterone (Bush, 1953a,b). However, Bush was unable to identify the active compound from the Wintersteiner amorphous fraction by paper chromatography, as he did not have a suitable bioassay. The obvious collaboration between Bush and our own group then occurred and it was rapidly demonstrated that electrocortin was secreted by dog and monkey adrenal glands. The active material in the Na^{24}/K^{42} assay from the adrenal blood extracts ran at the same speed in the Bush B5 system as the electrocortin obtained from the extracts of beef adrenal glands (Bush, 1952; Simpson et al., 1952).

Another important unique characteristic of the biological activity of electrocortin obtained from beef adrenal extracts was that it disappeared after acetylation but could be regenerated by alkali hydrolysis (with low but consistent yields by the methods used by us at the time) from a non-polar region of Bush paper chromatograms (Grundy et al., 1952; Simpson et al., 1952; Bush et al., 1953) (Fig. 5). The active compound in the dog adrenal blood behaved in the same manner. These properties were unique and constituted proof that electrocortin was secreted by mammalian adrenal glands and was not only present artefactually in adrenal extract. Later this was confirmed for dog adrenals by Farrell
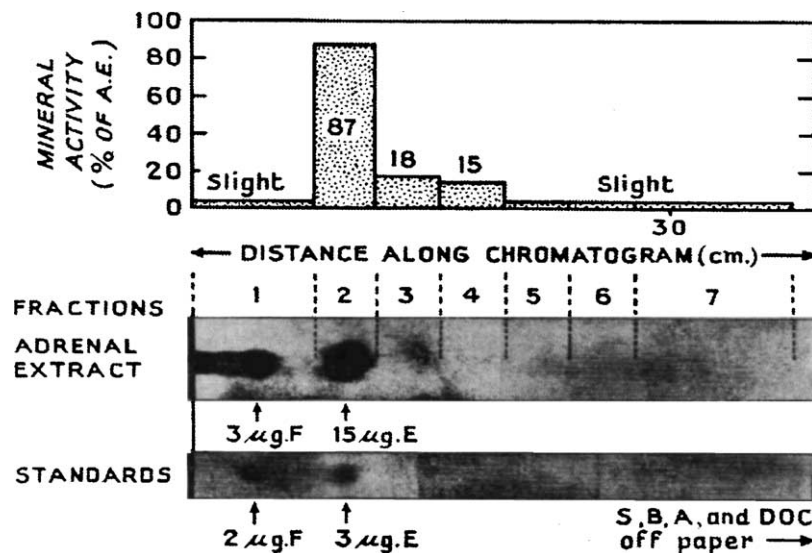

Fig. 3. Mineral activity of adrenal extract after running in Zaffaroni (pg-toluene) paper chromatographic system for 3 days (from Tait et al., 1952).
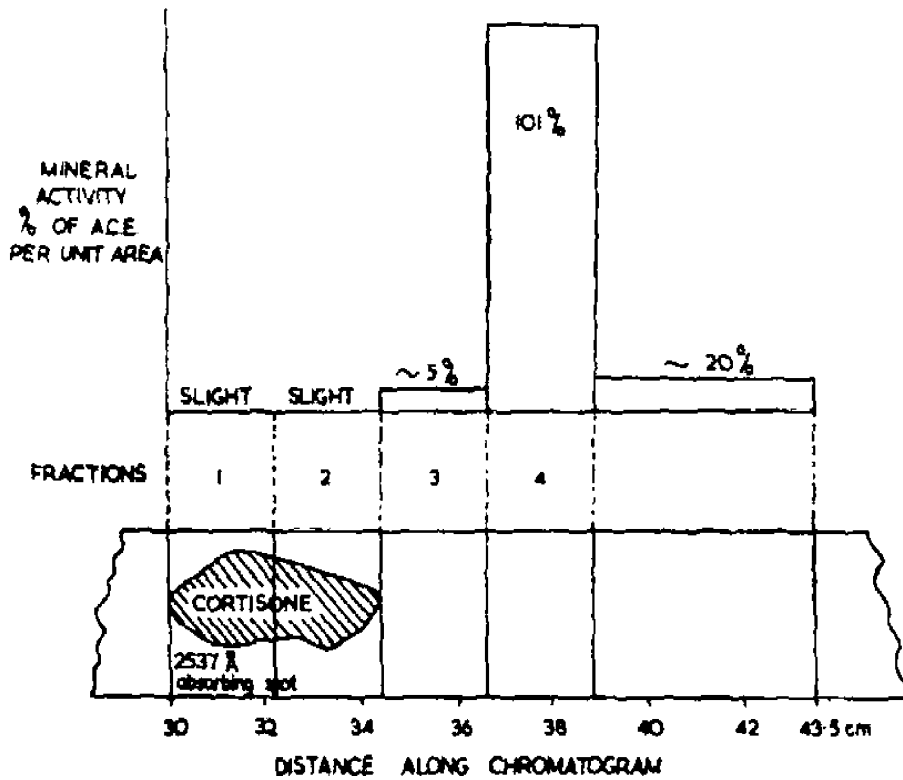
Fig. 4. Mineral activity of adrenal extract after running in Zaffaroni (pg-toluene) paper chromatographic system for 7 days (from Tait et al., 1952).

and co-workers (Farrell and Richards, 1953; Farrell et al., 1953; Farrell, 1955) and for rat adrenals by Singer and Stack-Dunne (1955).

John Luetscher and co-workers in the USA also obtained salt retaining activity with similar properties from the more difficult starting material of human urine (Luetscher et al., 1954). Relevant to the results of the RALES clinical trials (Pitt et al., 1999), aldosterone was first crystallised from

human urine obtained from two patients with congestive heart failure (Luetscher et al., 1956). In the earliest work of Luetscher's group, it was not known that the active compound originated from the adrenal gland. However, it soon became clear that the active compound in his material and our electrocortin were identical. Therefore, normal humans also secreted electrocortin and it was established as a general mammalian hormone from about 1952.
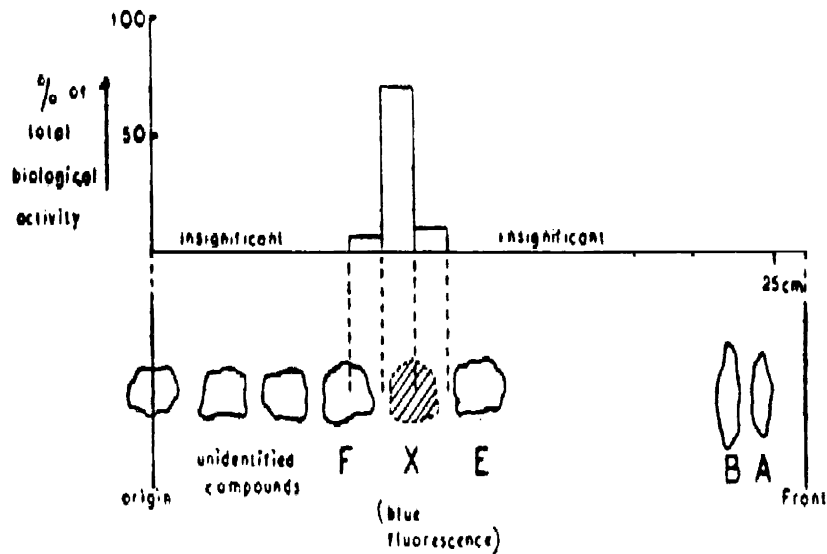
Fig. 5. Mineral activity of adrenal extract after running in Bush B5 (1:1 aqueous methanol–benzene) paper chromatographic system for 3 h. Steroids detected by soda fluorescence method. Biological activity from Na^{24}/K^{42} assay (from Simpson and Tait, 1953).
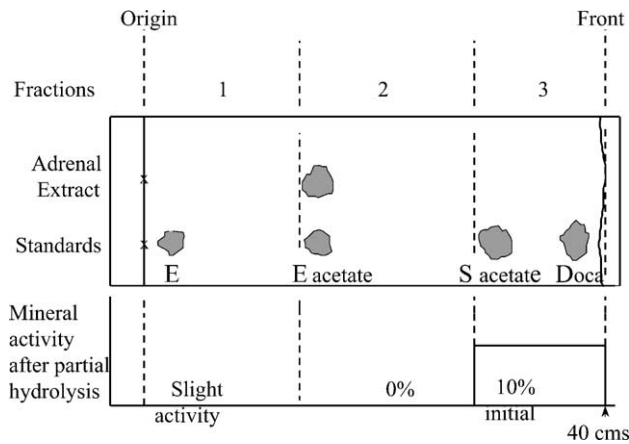
Fig. 6. Running properties of polyacetate in Bush 3 (aqueous methanol: benzene–petroleum ether) paper chromatographic system for 3 h. Electrocortin was acetylated with pyridine:acetic anhydride. The polyacetate was detected by mineral activity in Na^{24}/K^{42} assay after partial hydrolysis with alkali or acid (from Grundy et al., 1952a).

7. Crystallisation of electrocortin

The interest in the field then turned to the isolation in crystalline form, chemical identification and synthesis of electrocortin. The early workers in the adrenal field partitioned the adrenal extract in the solvent systems aqueous methanol:toluene or benzene. Butt et al. (1949) and Morris and Williams (1953) used similar systems for partition chromatography in kieselguhr columns pioneered 10 years earlier by Martin and Synge. These column methods were suitable for the preparation of larger amounts of the hormone (Fig. 6).

Morris generously taught us how to use such columns at maximum resolving power and we employed them to prepare 1 mg of material, which was as pure as the later crystalline electrocortin according to IR carried out by Dr. A.E. Kellie (MHMS). This also indicated that there was a Δ^4-3-oxo structure, so confirming the positive Bush soda fluorescence reaction (Bush, 1952; Simpson and Tait, 1953). Edwards at MHMS using a micromethod, oxidised some of the material and found it yielded formaldehyde quantitatively, so rigorously confirming the presence of the α-ketol group (Simpson and Tait, 1953). There was a weak but significant 20-oxo peak in the IR but no other oxo group showed, such as a free aldehyde (Simpson and Tait, 1953).

The polyacetate of aldosterone was also prepared and purified by column chromatography after acetylation using C^{14} carboxyl labelled acetic anhydride (Fig. 7). From its specific activity, it was concluded that there were two acylable hydroxyl groups. According to the infra red spectroscopy (IR), there was no hydroxyl group after full acetylation. It, therefore, seemed certain that the molecule of electrocortin had a Δ^4-3-oxo and an α-ketol group. Also, there were two acylable hydroxyl groups, one at 21 and one at an unknown position (Simpson and Tait, 1953).
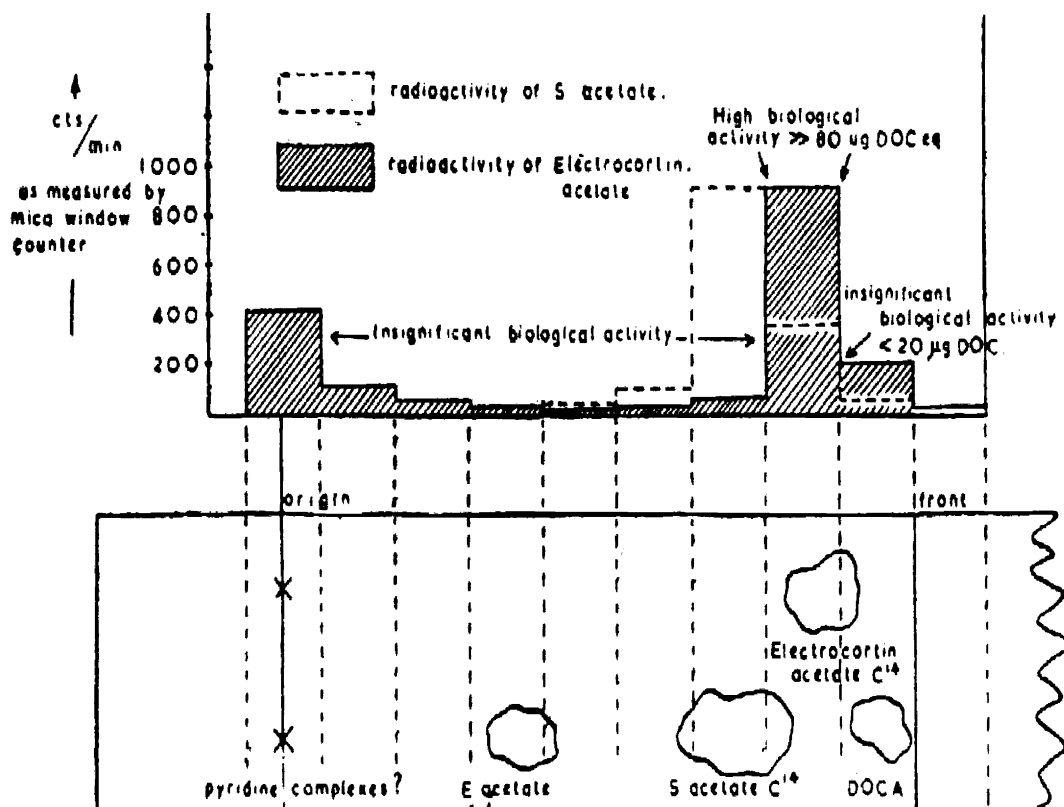

Fig. 7. Running properties of electrocortin C^{14} diacetate in Bush B3 paper system (from Simpson and Tait, 1953).

At the time, we tentatively suggested that the unknown acetylated group in the diacetate could be at position 16 (Simpson and Tait, 1953). The identification of aldosterone by Reichstein showed that the structure did not have a 16 hydroxyl, although he seriously considered this possibility at one stage (Tait and Tait, 1998b). Ironically, it was eventually found that the substitution of steroids at 16, e.g. triamcinolone, led to the loss of the salt retaining activity of the parent molecule.

8. Isolation in crystalline form

At this point, it became clear that greater quantities of material for more classical degradation studies were necessary. We then started full collaboration with Reichstein (and the Ciba group, including Drs. A. Wettstein, R. Neher and A. Schmidlin) and supplied them with all our information on the properties of electrocortin (Fig. 1). Reichstein and von Euw then started the purification of the extract (by the Cartland and Kuizenga procedure) from 500 kg of beef adrenals prepared by Organon Ltd., Holland. They used partition chromatography on large kieselguhr columns with aqueous methanol:benzene solvent systems and eventually 21 mg of electrocortin was crystallised. However, this was not achieved without serious difficulties.

Reichstein did not routinely use the Na^{24}/K^{42} bioassay to control the separations. The column fractions were only analysed by paper chromatography by assistants who were not experienced with electrocortin. A compound in the column fractions was followed, which turned out not to be electrocortin and at one point it was thought that all the biologically active compound in the original extract, had been lost (Tait and Tait, 1998b). This was a tense situation as the preparation of the extract was extremely costly and it was not thought that another purification run would have been possible. The unknown compound, mistakenly taken for electrocortin, was in fractions less polar than cortisone. We did not expect this if the process was one of pure partition chromatography with no influence from the support, which seemed likely. After some correspondence between Reichstein and ourselves, a biologically active compound identical with electrocortin was found in some of the fractions in the position expected by us and more polar than cortisone (but less polar than cortisol). This material was crystallised by Reichstein (Simpson et al., 1954a,b; Tait and Tait, 1998b).

This 21 mg of crystalline electrocortin was sufficient in quantity for degradative studies, even at that time, at least in the hands of a steroid chemist as skilled as Reichstein. Shortly afterwards, smaller quantities were also crystallised in the laboratories of the Ciba team and our own. Also, the Mayo group crystallised the hormone within a few weeks of Reichstein (Mattox et al., 1953a,b). Although this has been described as a race between the Reichstein and Mason groups, the Mayo Clinic team were closely following and adopting the methods used by us, including the elec-

trolyte ratio bioassay and the chromatographic procedures. However, we could hardly complain about the activities of a group, who had been working with the amorphous fraction since 1935. In the following year, the Merck group also succeeded in obtaining crystals of the hormone (Harman et al., 1954).

9. Identification and synthesis

Apart from in the original publications, the degradative studies conducted by Reichstein have been described and discussed extensively elsewhere (Wettstein, 1954; Neher, 1979; Fieser and Fieser, 1959; Tait and Tait, 1998b). There is agreement that the crucial compound was the oxidation product of the free electrocortin (a γ-lactone, III, Fig. 7) which sublimed and crystallised easily and could be purified directly from crude preparations (Wettstein, 1954). It was concluded that there was an aldehyde group at the 18 position mainly from the properties of this oxidation product. It was also concluded that there was a hemiacetal formed from the 18 aldehyde (e.g. the from the compound after oxidation of the aldosterone 21-monoacetate). Our investigation, previously described, using C^{14} acetic anhydride indicated that the acetyl derivative was a diacetate (rather than a triacetate that had been suggested by preliminary results in Basle). This was a critical issue at one stage of the work. If there were two acylable hydroxyl groups (one presumably at position 21) and no remaining hydroxyl groups, then a hemiacetal was formed with a hydroxyl group at positions 8, 11, 15 or 16 (Simpson and Tait, 1953; Wettstein, 1954; Fieser and Fieser, 1959; Neher, 1979; Tait and Tait, 1998b). Reichstein, who had first favoured the 15 or 16 position, suddenly and intuitively correctly chose the 11β position and he was proved to be correct after further classical chemical studies (Tait and Tait, 1998b) (Fig. 8).

During this critical stage, we sent a sample of our pure electrocortin to R. Speirs in Bar Harbor who had a mouse eosinophil test, which sensitively measured glucocorticoid effects (Speirs and Meyer, 1953). We simply intended to confirm our assumption that electrocortin did not possess appreciable glucocorticoid activity. To our surprise, electrocortin was found to be about one quarter as active as cortisone in the mouse eosinophil test (Speirs et al., 1954). At the time, Reichstein had started to consider the possibility that aldosterone had an 11β-hydroxyl group but we were not aware of this when we sent the sample of electrocortin to Speirs. Reichstein regarded the results of the eosinophil test as being in support of his ideas on the structure, although this was probably not a critical factor in his considerations (Tait and Tait, 1998b). The function of the glucocorticoid activity of aldosterone (if any) has still to be discovered as also the possible relevance of the report of a high incidence of NIDDM in primary aldosteronism (Conn et al., 1964).

Finally, in 1954, the structure of aldosterone was elucidated as 11β-21-dihydroxy-18-oxo-pregn-4-ene-3,20-dione
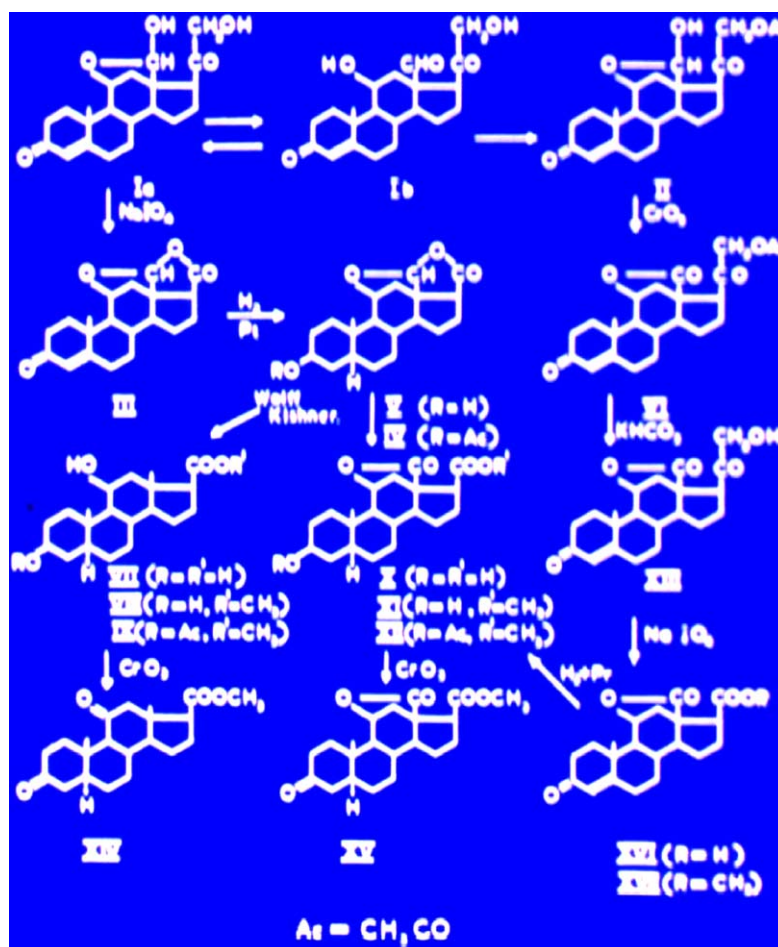
Fig. 8. Degradation scheme used by Reichstein. Key use of lactone (III) (from Simpson et al., 1954a).

by Reichstein and co-workers (Simpson et al., 1953b, 1954b; Fig. 9). In solution, it can exist in three tautomeric forms, as shown here (Fig. 10) which is from the paper by Neher (1979) presented at the corresponding 25th Anniversary Symposium. This tautomerism explains the lack of the aldehyde peak and the weak 20-oxo peak in the IR absorption spectra, which we (i.e. Dr. A. Kellie) first observed and was confirmed with the crystalline material (Tait and Tait, 1998b). Clearly, Reichstein was the key scientist involved in the final identification of aldosterone and he appropriately renamed electrocortin, aldosterone. Soon afterwards the Mayo (Mattox, 1955) and Merck (Ham et al., 1955)
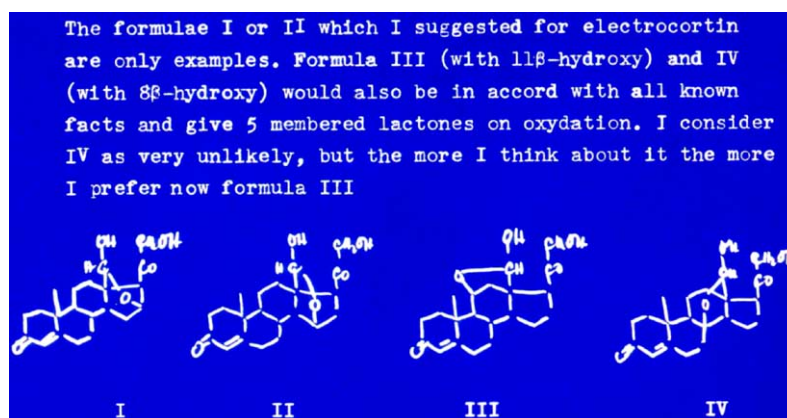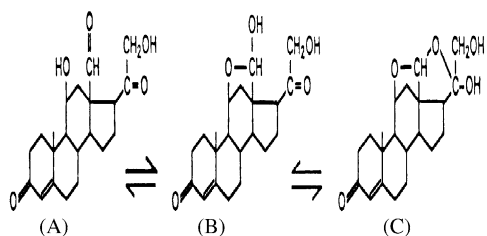

Fig. 9. Reichstein intuition on structure of aldosterone. Original correspondence of S.A.S. Simpson and J.F. Tait with T. Reichstein. Welcome Museum for the History of Science, London (Tait and Tait, 1998a).

from Neher R. Symposium on Aldosterone. 25th Anniversary.

Fig. 10. Tautomers of aldosterone (from Neher, 1979).

groups confirmed this structure. Aldosterone was first synthesised by the Ciba group in Basel (Schmidlin et al., 1955; Vischer et al., 1956) and subsequently by Reichstein and also chemists at Organon (Fieser and Fieser, 1959; Neher, 1979). Later, an original method of synthesis by Barton involved photolysis of corticosterone acetate nitrite to aldosterone acetate oxime, which could then be readily converted to aldosterone 21-monoacetate (Barton et al., 1961).

10. Metabolism of aldosterone

The unexpected structure of aldosterone contained both an aldehyde at position 18 and an 11β-hydroxyl group with an 11–18 hemiacetal preferentially formed in solution (Simpson et al., 1953b, 1954b; Neher, 1979, Fig. 10; Tait and Tait, 1998b). This structure gave aldosterone unique properties, both chemically and biologically, as was clear when its metabolism was studied.

The 18-hydroxyl group in the hemiacetal structure is available for metabolism in humans as in the formation of aldosterone 18-glucuronide, as first found by Underwood in our group at the WFEB (Underwood and Frye, 1972; Underwood and Tait, 1961, Fig. 11). Carpenter and Mattox
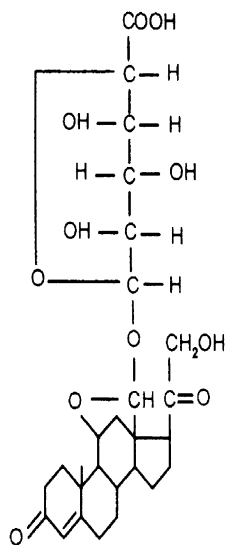

Fig. 11. Aldosterone 18β-D-glucosiduronic acid (aldosterone 18-glucuronide). (Underwood and Tait, 1961; Underwood and Frye, 1972).

(1976) later confirmed the structure more completely and rigorously as the β-glucosiduronic acid. This is the urinary metabolite of aldosterone, first measured by Luetscher (1953), and later found by his group to be formed to a major extent in the kidney. It is hydrolysed more readily in acid conditions than other glucuronides, such as those on the 3-position, but not so readily enzymatically in vitro, e.g. β-glucuronidase, and probably also in vivo (Underwood and Tait, 1961; Carpenter and Mattox, 1976). It seems that the aldosterone 18-glucuronide is fairly stable in the body (Fig. 11). The possibility that it might be biologically active was an interesting possibility at one time. Relevantly, acetylation of the 18-hydroxyl group could occur (as for aldosterone 18-monoacetate and 18,21-diacetate) even under mild conditions. These 18-acetates, unlike the 21-monoacetates, are relatively biologically fairly inactive; a factor which hindered the early work of Reichstein on aldosterone as previously mentioned. This is probably because the 18-monoacetate, like the 18-glucuronide, is not hydrolysed readily in vivo enzymatically.

We and others studied the metabolism of aldosterone (and other steroids) in humans using ^{3}H and ^{14}C labelled steroids prepared in collaboration with W. Pearlman in London (Ayres et al., 1958a) and Marcel Gut at the Worcester Foundation. These chemists synthesised radioactive progesterone and this was converted biosynthetically by rat capsular strippings to labelled aldosterone by our team. We presented some of the ^{3}H aldosterone made at the WFEB to the US Endocrine Study Section and it was rewarding to see it used generally. This was typical of the late Marcel Gut's many unselfish contributions in supplying radioactive steroids in the field.

It was soon found that there was a very high (nearly 100%) hepatic extraction of aldosterone in normal subjects as determined after oral and intravenous administration of ^{3}H and ^{14}C aldosterone, and/or hepatic blood sampling (Tait et al., 1965; Flood et al., 1967). The metabolic clearance rate of aldosterone was found to be high compared to that of other adrenal steroids, such as cortisol and corticosterone (Tait et al., 1961; Tait and Burstein, 1964; Tait and Tait, 1998a). This was mainly because of the weak binding of aldosterone to circulating binding high affinity proteins, such as CBG (Meyer et al., 1961) and the resulting nearly 100% hepatic extraction (Tait et al., 1965). The extrahepatic conversion to the 18-glucuronide also contributes to the high MCR to a certain extent (Bledsoe et al., 1966; Cheville et al., 1966). This high MCR of aldosterone means that the inertia of its metabolic system is low and changes in circulating blood concentration follow rapidly those in secretion rate. This is in contrast to cortisol, which, at normal concentrations, has a low metabolic clearance rate and high metabolic inertia due to its strong binding to CBG (Tait and Burstein, 1964). Recent findings on the action of aldosterone have shown that the intrinsic rate of actions (both non-genomic and even genomic) are fairly rapid (Christ et al., 1999). Therefore, fast changes in the blood concentrations of aldosterone may

result in rapid action. For cortisol, a more constant level may be appropriate for a 'permissive' type action.

Even more interesting is it seems that the 11–18 hemiacetal formation actually protects the 11β-hydroxyl group in aldosterone from metabolism (Ulick et al., 1979; Stewart et al., 1990). The Type 1 mineralocorticoid receptor in kidney tubules has about an equal affinity for aldosterone and other adrenal steroids, such as cortisol and corticosterone (Funder, 2001). However, according to current theories, aldosterone remains the dominant mineralocorticoid because other potentially competing 11β-hydroxy steroids binding to the same receptor are converted to the corresponding inactive 11-oxo steroids, such as cortisone, by an 11β-dehydrogenase. However, the 11β-hydroxyl group of aldosterone is protected presumably because of the hemiacetal structure.

Therefore, the 18-hydroxyl of aldosterone is available for metabolism but the 11β-hydroxyl group is protected. However, although theoretically the two processes work in opposite directions on aldosterone levels, it is unlikely that these are competing routes of overall metabolism in determining circulating aldosterone concentrations.

11. Adrenal site of production of steroids

As regards the classical question as to the purpose (if any) of the division of the adrenal into the zona glomerulosa, fasciculata-reticularis (Fig. 12), there had been various theories.

One type (the functional zonation or zonal hypothesis) suggested that the various zona produced different steroids;
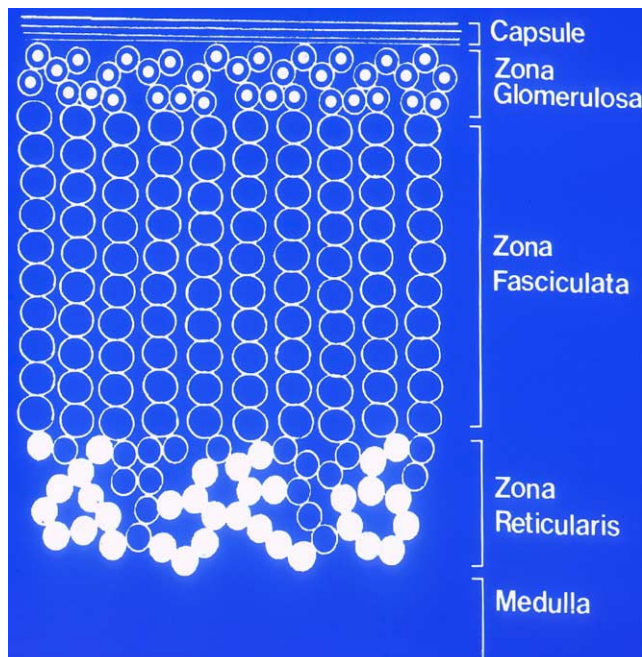
Fig. 12. Diagramatic histology of adrenal cortex.

Fig. 13. Theories of the function of zonation of adrenal cortex.

the other (the cellular migration hypothesis) that these were regions of different mitotic activity (Fig. 13).

The discovery of aldosterone meant that the functional zonation theory could be investigated more directly. Giroud et al. (1956) in Montreal, demonstrated that the zona glomerulosa of the rat exclusively produced aldosterone, so confirming the classical but more indirect studies of Swann (1940) and Deane et al. (1948). At about the same time, Ayres in our group also found exclusive production of aldosterone in both the rat and beef zona glomerulosa of adrenal glands and of cortisol in the zona fasciculata-reticularis in the beef adrenal (Ayres et al., 1956). These results supported the functional zonation hypothesis.

We also found preferential production of aldosterone by the zona glomerulosa and cortisol by the zona fasciculata-reticularis of human adrenal glands although because of their more convoluted nature this is more difficult to demonstrate than in rat and beef glands (Ayres et al., 1958b). However, Fig. 14 clearly shows aldosterone decreasing from the outer (zona glomerulosa) to the inner (zona fasiculata) slices and cortisol (and cortisone) correspondingly increasing.

Later, a rather more complex picture emerged for other 18 oxygenated steroids (Fig. 15), such as 18-hydroxy corticosterone (produced mainly but not exclusively by the glomerulosa) and 18-hydroxy deoxycorticosterone (mainly by the zona fasciculata) which no doubt will be dealt with in other papers in the meeting.

12. Biosynthesis of aldosterone

After the identification of aldosterone, the pathways to the biosynthesis of the hormone were studied (Wettstein, 1954; Ayres et al. 1956, 1960, 1970) (Fig. 15). As expected from the structure of aldosterone, deoxycorticosterone and progesterone were soon found to be precursors. We also found (with Peter Ayres) using ox adrenal strippings that corticosterone was an intermediate in a major pathway to aldosterone (Fig. 16). This was perhaps also not unexpected from the structure of aldosterone. However,
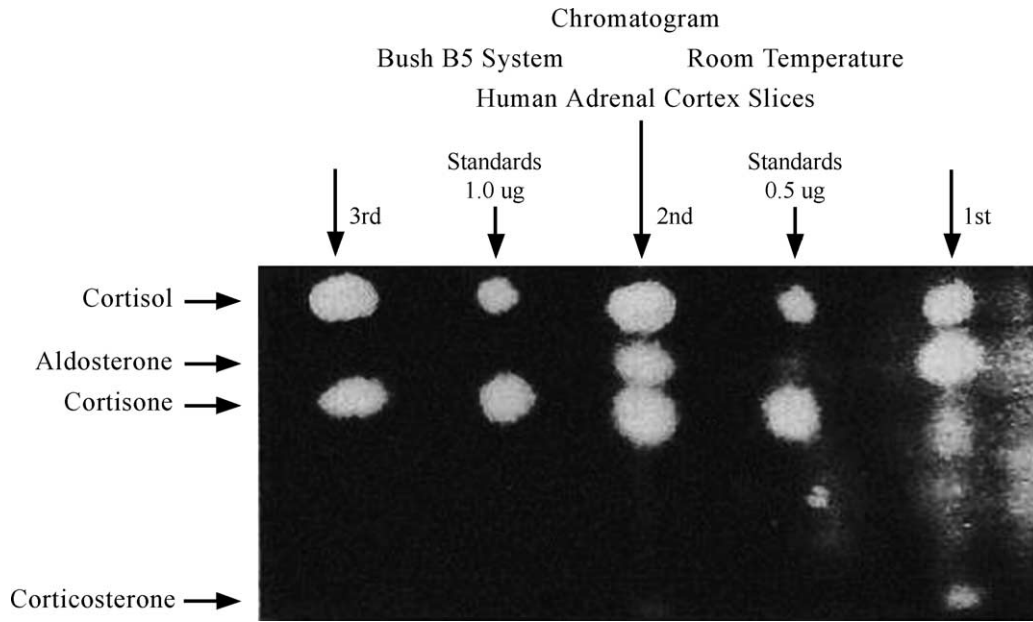
Fig. 14. Production of aldosterone, cortisol and cortisone by human adrenal cortex slices (from Ayres et al., 1958b).
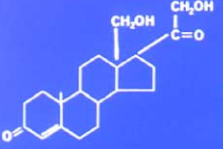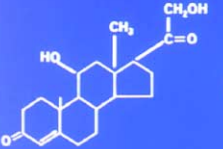

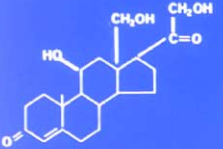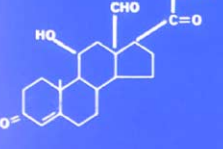Fig. 15. Site of production of 18-hydroxy steroids by zones of the rat adrenal cortex.
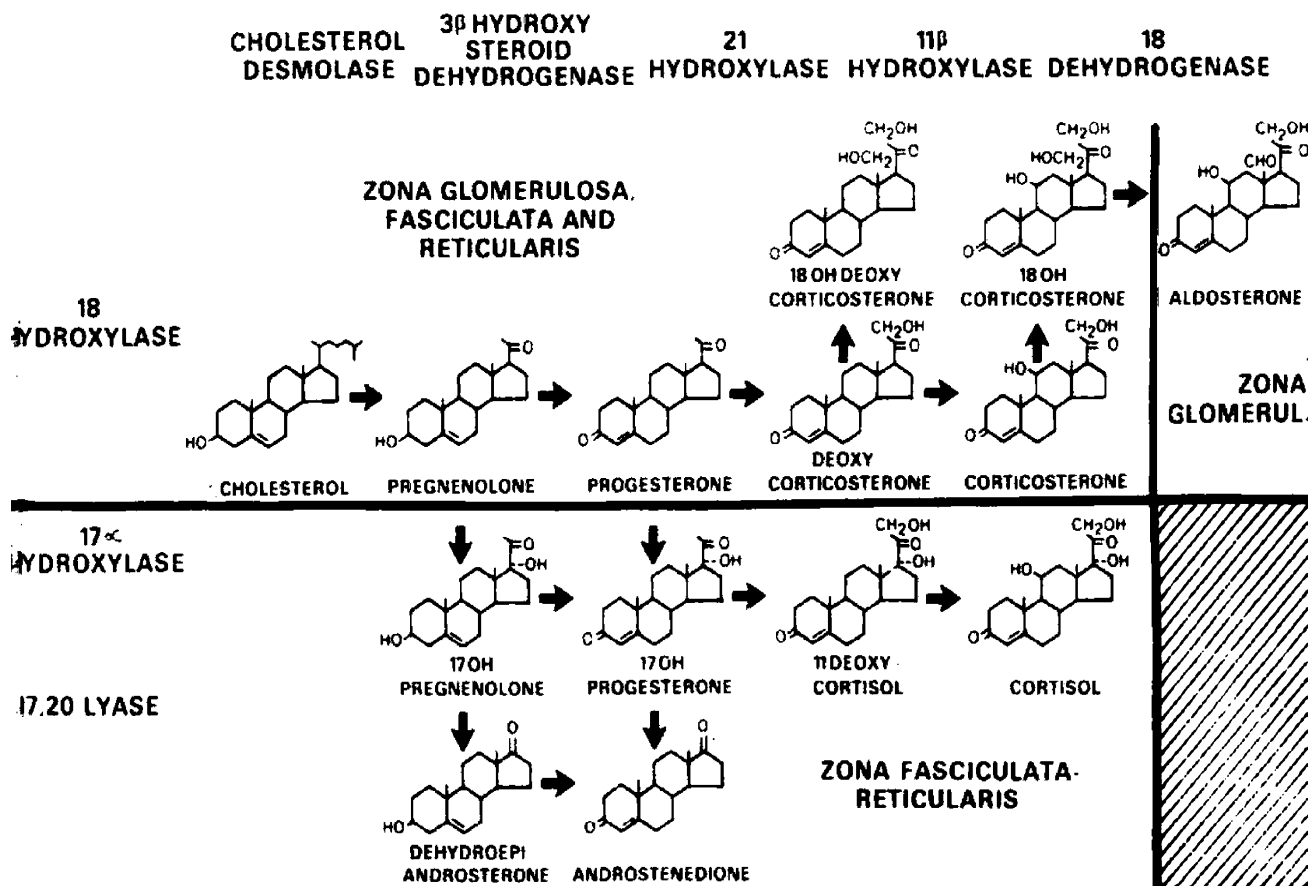
Fig. 16. Biosynthesis of aldosterone with corticosterone as intermediate.

Oscar Hechter in his classical studies of the biosynthesis of corticoids had just concluded that 'the adrenal enzymes regarded 11β-hydroxylation as the trade mark of an end product' (Hechter and Pincus, 1954; Wettstein, 1954; Kahnt and Neher, 1965; Ayres et al., 1956, 1960, 1970; Neher, 1970).

The use of the sheep adrenal transplant preparation in Melbourne, Australia, then gave us the opportunity to study the biosynthesis of aldosterone under in vivo conditions of stimulation of secretion, an approach also used by Eik-Ness and Kekre (1963) for the in vivo testes. It was found that under conditions of mild salt depletion, the pathway through corticosterone was probably the most important (Blair-West et al., 1970; Fig. 17).

Subsequently, the possible importance of 18-hydroxy deoxycorticosterone as an intermediate in the biosynthesis of aldosterone, particularly under conditions of severe salt depletion, has been raised (Vinson et al., 1995; Boon et al., 1996) (Fig. 18). It feels rather strange to us that the pathway through corticosterone is now the 'classical' route and that through 18-hydroxy deoxycorticosterone is considered to be the alternate route. Before we demonstrated that the pathway through corticosterone to aldosterone was of major importance, this was considered to be an unlikely alternate pathway.

13. Clinical studies

In the clinical field, a great deal of effort has been spent over the last 50 years, including by our group, in devising accurate methods of measuring aldosterone levels in humans. At first sight, therefore, it has been rather disappointing when there has been a lack of correlation of measured aldosterone levels with the expected clinical effects of the hormone. The following are the examples of this.

13.1. Congestive heart failure

Although Luetscher and co-workers (Luetscher et al., 1956) crystallised aldosterone from the urine of two patients with congestive heart failure and oedema they have reported, that many such cases showed normal excretion of aldosterone 18-glucuronide (Deming and Luetscher, 1950). Sanders and Melby (1964) found that the urinary excretion of tetrahydroaldosterone or the secretion of aldosterone was not higher than normal cases in most of the 10 cases of congestive heart failure they studied. Our group reported that there was lowered metabolic clearance rate of aldosterone in heart failure correlating with the severity of the condition (Bougas et al., 1964; Tait et al., 1965). From direct measurements of hepatic venous blood, this was found to be usually
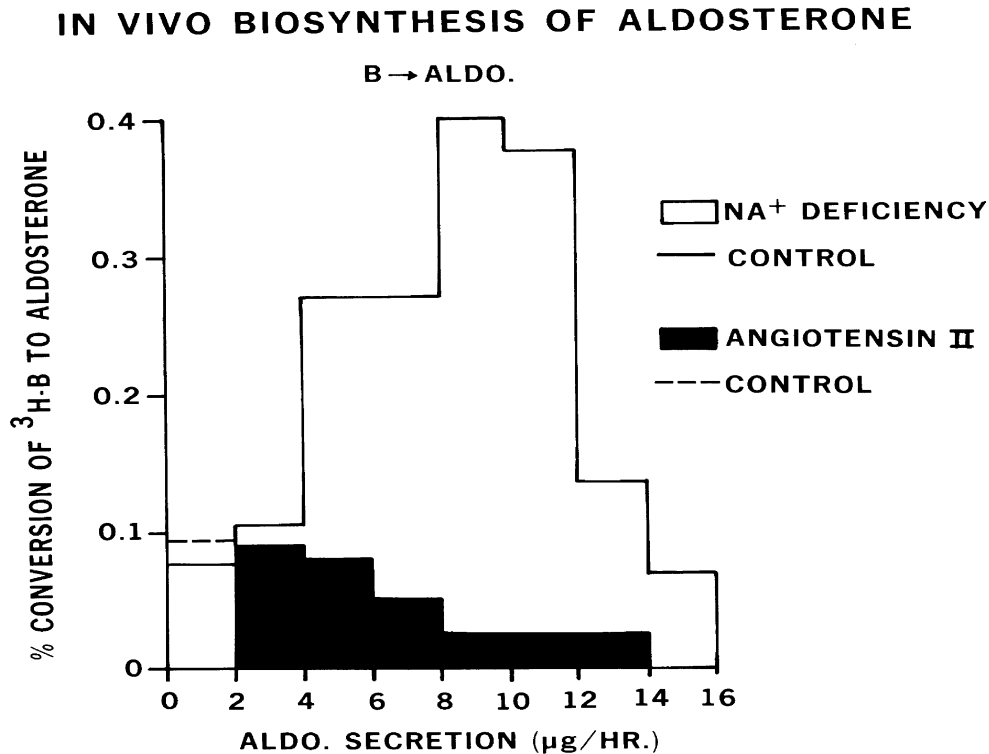
IN VIVO BIOSYNTHESIS OF ALDOSTERONE

Fig. 17. Effect of Na depletion on conversion of corticosterone to aldosterone in sheep adrenal transplant (from Blair-West et al., 1970).

due to lowered hepatic extraction of aldosterone but in the severest cases sometimes also decreased hepatic blood flow. Luetscher and co-workers (Camargo et al., 1965; Cheville et al., 1966) confirmed these findings and also reported that only the excretion of metabolites but also the secretion of aldosterone were normal in many of these patients. However, if the metabolic clearance rate of aldosterone were lowered, the peripheral plasma concentration of the hormone would be increased so explaining the biological effects even with normal secretion rates of aldosterone. In confirmation of

Fig. 18. Alternate biosynthetic pathways to aldosterone (from Boon et al., 1996).

this, Sanders and Melby (1964) showed that 40% of all their 10 cases of congestive heart failure exhibited a negative sodium balance (loss of weight) with spironolactone and all these had normal excretion of aldosterone metabolites).

In the accompanying Editorial after the publication of the results of the RALES trials in the *New England Journal of Medicine* (Pitt et al., 1999) demonstrating that spironolactone can markedly improve the prognosis of patients with congestive heart failure, Weber pointed out the possible importance of the lowered MCR of aldosterone in causing the unexpected 'escaped' levels of the plasma concentration of aldosterone after administration of ACE inhibitor (Weber, 1999). There does not seem to have been any study to measure the relative effects of increase in secretion and lowering of MCR on the plasma concentration of aldosterone in this situation. However, it is possible that effects of stimuli of aldosterone secretion, such as increased plasma concentration of K^+, may maintain the aldosterone secretion and this effect can be amplified by the lowered MCR of aldosterone. Increased circulating aldosterone may cause heart fibrosis (Brilla, 2000; Lijnen and Petrov, 2000).

The really surprising result of the RALES trials was the small dose of spironolactone which was effective therapeutically (Pitt et al., 1999). This dose did not cause natriuresis so that aldosterone may not be measured in the appropriate site and alterations in the general MCR and the circulating aldosterone may be irrelevant. There has been a suggestion that autocrine effects of locally produced aldosterone in the heart may be important although this is not yet established
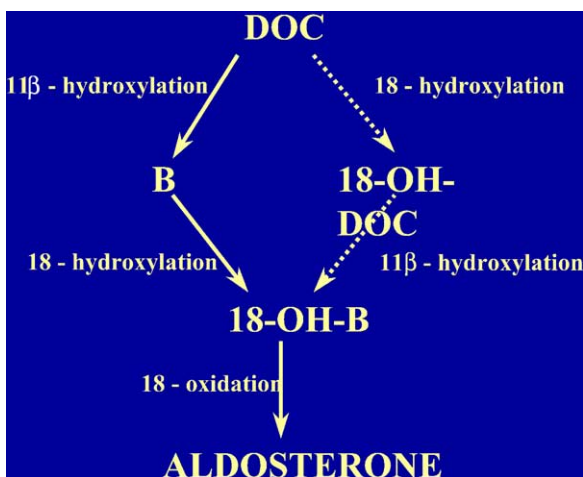
(Delcayre and Silvestre, 1999; Delcayre et al., 2000). In this connection, the results of Coghlan et al. (1971) may be of interest. They found that, as measured by a specific method, the plasma concentrations of aldosterone in five patients with heart failure and oedema were not increased. This does not agree with the calculated values for peripheral blood concentrations of aldosterone of Luetscher which were mostly above normal (Camargo et al., 1965). If the values of Coghlan et al. are correct this would suggest that general peripheral blood concentrations may not be the appropriate site for measurements of clinically effective aldosterone in heart failure. Theoretically, steroid dynamics indicates that this could be the case if there is significant production of aldosterone in an outer compartment, e.g. the heart. However, it could still be that peripheral plasma concentrations are a good guide to patients whose, for one reason or another, aldosterone secretion has escaped from the effect of ACE inhibition and, therefore, may respond to aldosterone inhibitors. Nevertheless, there is no doubt that the measurement of a urinary metabolite or even secretion rate of aldosterone may be misleading in this condition as regards the effects of the hormone. Recently, Young et al. (1999) and other groups have found that there are opposing effects on cardiac hypertrophy of mineralocorticoid (aldosterone and deoxycorticosterone) or glucocorticoid (9α-fluorocortisol) receptor occupancy in rats.

13.2. Primary aldosteronism

In the first three cases of primary aldosteronism that we studied in the 1950s, the urinary excretion of aldosterone 18-glucuronide was found to be only in the upper normal range (about 2–3× normal mean excretion); less than found for many cases of secondary aldosteronism (Ayres et al., 1958). These determinations were by an accurate physico-chemical method. However, primary aldosteronism would certainly not have been discovered as a consequence of our measurements in such cases. One of these was the second one encountered by Milne of Hammersmith (Ayres et al., 1958b; Milne and Muehecke, 1956). We reported to him that the aldosterone 18-glucuronide excretion of the patient was only about twice normal. Shortly afterwards, Prof. Milne entered our laboratory with a dish containing the small but definite adrenal adenoma removed from the patient. He made a short statement, which translated politely meant 'so much for your urinary measurements'. On incubation, this adenoma produced moderately high aldosterone and corticosterone production and appreciable but low cortisol. It did not have the zonal production characteristic of normal human adrenal tissue (Ayres et al., 1958b). Several other cases of primary aldosteronism we studied also had urinary aldosterone only in the upper range of normal. On the other hand, the first case of Milne, which first presented in 1953 (Milne and Muehecke, 1956) and that of Conn (1955) had high aldosterone excretion according to bioassays. We now know from the studies of several leading groups in the field that aldosterone excre-

tion is not always greatly increased in patients with primary aldosteronism compared with patients with normal electrolyte metabolism (Gomez-Sanchez, 1999; Streeten et al., 1979). It seems that the appropriate controls are actually subjects with similar plasma K^+ and angiotensin II concentrations, which have a much lower aldosterone excretion. It seems that that the production of aldosterone by the remaining 'normal' adrenal tissue is depressed nearly completely (and perhaps irreversibly). In two of our early cases, the production of aldosterone post-operatively remained very low for many months. On the other hand, adenoma tissue from all the cases we studied produced substantial amounts of aldosterone in vitro (Ayres et al., 1958b). The adenomas seem to be autonomous, at least to some extent, to the effects of lowered K^+. It seems that the first cases of Milne and Conn, showing substantial excretion of aldosterone, had unusually large adenomas (4 cm), whereas the adenoma from our three cases, showing relatively low excretion of aldosterone, had adenomas of about 2 cm. According to Conn's analysis of 106 cases, this is about the average size (Conn et al., 1964).

In our early studies, we examined other possible reasons for the relatively low extent of the raised excretion of aldosterone in primary aldosteronism, such as the existence of excess amounts of other mineralocorticoids (Garrod et al., 1956; Ayres et al., 1958b). However, corticosterone, which is a candidate, for this role is only a weak mineralocorticoid. We also found that there was not abnormal conjugation of urinary metabolites (aldosterone 18-glucuronide/free aldosterone). Recently, Gomez-Sanchez (1999) has suggested that the unexpectedly low urinary aldosterone generally found now in primary aldosteronism be due to the measurement of a 'minor' urinary metabolite, aldosterone 18-glucuronide. However, because the final measurement is on the free aldosterone after hydrolysis of the 18-glucuronide does not mean that aldosterone 18-glucuronide itself is a 'minor' metabolite. It would be unexpected if there were to be a change in the pattern of metabolites similar to that seen in hepatic cirrhosis with such small changes in the secretion of aldosterone in primary aldosteronism. However, we await publication of the full data of Gomez-Sanchez (1999) on this topic with great interest.

Meanwhile, the most likely explanation of the unexpectedly relatively low excretion of aldosterone in primary aldosteronism is that the secretion of aldosterone is inappropriately raised for the prevailing electrolyte situation. This is usually accompanied by a low circulating renin hence the recent diagnostic success of the plasma aldosterone/renin activity value for primary aldosteronism. Relatively low aldosterone excretion probably helped to delay recognition of the high frequency of primary aldosteronism in hypertension. In any case, it seems that in primary aldosteronism, the poor correlation of the absolute levels of aldosterone and its clinical effects is due not to the measurement of an unsuitable quantity, e.g. metabolite excretion rather than secretion, but to the choice of unsuitable control subjects with a different

electrolyte status. The appropriate controls would be normal subjects with a low circulating plasma K^+ concentration.

13.3. Aldosterone and diabetes

Recently, the Taits especially, were interested in another clinical situation where the correlation of aldosterone levels and the clinical effects are not straightforward. There seems to be a disturbance of the RAAS system in NIDDM with some patients having an inappropriately 'normal' level of aldosterone in spite of the salt retaining effects of increased insulin. Insulin seems to be intrinsically and directly salt retaining, as was shown by DeFronzo et al. (1975) and others from in vivo experiments in humans. It probably acts directly on the thick ascending loop of Henle, according to some of these investigators. The recent in vitro work of the action of aldosterone and insulin on SGK by Pearce and co-workers (see Wang et al., 2001) would also support a direct action of insulin, at least if their results can be applied to the in vivo situation. It might be relevant that there is a correlation of insulin levels and blood pressure in people of the same race which could be explained if insulin is intrinsically salt retaining (Tait and Tait, 1996).

A Swiss team of De Chatel et al. (1977) found that in a group of early NIDDM patients there was a marked increase in total body sodium but a 'normal' absolute level of blood aldosterone using a specific method. The nearly normal levels of aldosterone in most early NIDDM patients has been more recently confirmed as reviewed by Flack et al. (1998), although there is a sub-group of patients with low levels of renin and aldosterone. Plasma insulin is clearly increased in most early NIDDM patients. If this insulin is active on electrolyte metabolism intrinsically, a fall in aldosterone levels might have been expected after the marked increase in body sodium. This would have counteracted, to some extent, the primary effects of the increased insulin. Therefore, we have suggested that there must be some extra stimulus to aldosterone secretion to maintain the 'normal' levels of blood aldosterone in early NIDDM (Tait and Tait, 1996). It has been proposed by Goodfriend et al. (1993, 1998) that such stimulation could be through the effects of increased levels of active hepatic products of fatty acids, such as linoleic acid. Also, there may be a role for amylin, co-secreted with insulin, as proposed by Cooper et al. (1995). Amylin and insulin productions are both increased in early NIDDM. Reasonable amounts of amylin can markedly stimulate renin production in rats and humans according to Cooper et al. (1995) although perhaps the results in man need to be confirmed using lower doses of amylin.

More recently, Epstein (2002) has found that eplerenone and an angiotensin converting enzyme inhibitor, enalapril, can be equally effective in lowering blood pressure in NIDDM patients. This again indicates, as does the unexpectedly 'normal' blood concentrations of aldosterone, that this hormone probably has some effect in promoting hypertension in NIDDM. In this study, eplerenone was more effective than enalapril in decreasing albuminuria but that is another story.

This hypothesis regarding the hypertension of NIDDM could be regarded as inappropriate hypersecretion of aldosterone under the prevailing conditions as for certain cases of primary aldosteronism. For early NIDDM patients, the appropriate control subjects would presumably be normal subjects infused with pure insulin.

13.3.1. Species differences in steroid secretion

There is one puzzle in the adrenal field which still intrigues us. As previously mentioned, just before the work on aldosterone secretion, Bush (1953b) found a marked species differences in the cortisol to corticosterone ratio in adrenal venous blood (Fig. 18). This ratio ranged from about 20 for primates to <0.05 for rats. The functional reason, if any, for this species difference was not clear except in the guinea pig where increased transcortin binding alters cortisol dynamics (Keightley et al., 1988). Studies on the aldosterone secretion in various species might have been expected to throw some light on this question, e.g. did corticosterone take over the role of the major mineralocorticoid from aldosterone in some species such as rodents? However, it was found that all mammals secrete aldosterone in biologically significant quantities with no really marked difference between species except in frogs where the aldosterone, and 18-OH corticosterone is very high. Therefore, if there were a physiological significance to the differences in the ratio of cortisol/corticosterone secreted, it seems that it was unlikely to be connected with effects on electrolyte metabolism but rather on some aspects of their actions on carbohydrate metabolism. It may be that primates require a higher ratio of glucocorticoid to mineralocorticoid activity to be secreted by the adrenal than that provided by corticosterone as the glucocorticoid. The reason for this possible requirement is not clear but there are several possibilities, such as the need to prevent fibrotic or other pathological changes in the heart and other organs in primates (Table 2). Conn and co-workers showed a lack of effect of corticosterone in the treatment of patients with rheumatoid arthritis compared with those of cortisol using doses of corticosterone which would have been expected to have similar effects on general carbohydrate metabolism (Bush, 1952). Therefore, corticosterone is

Table 2
Species differences in adrenocortical steroid production

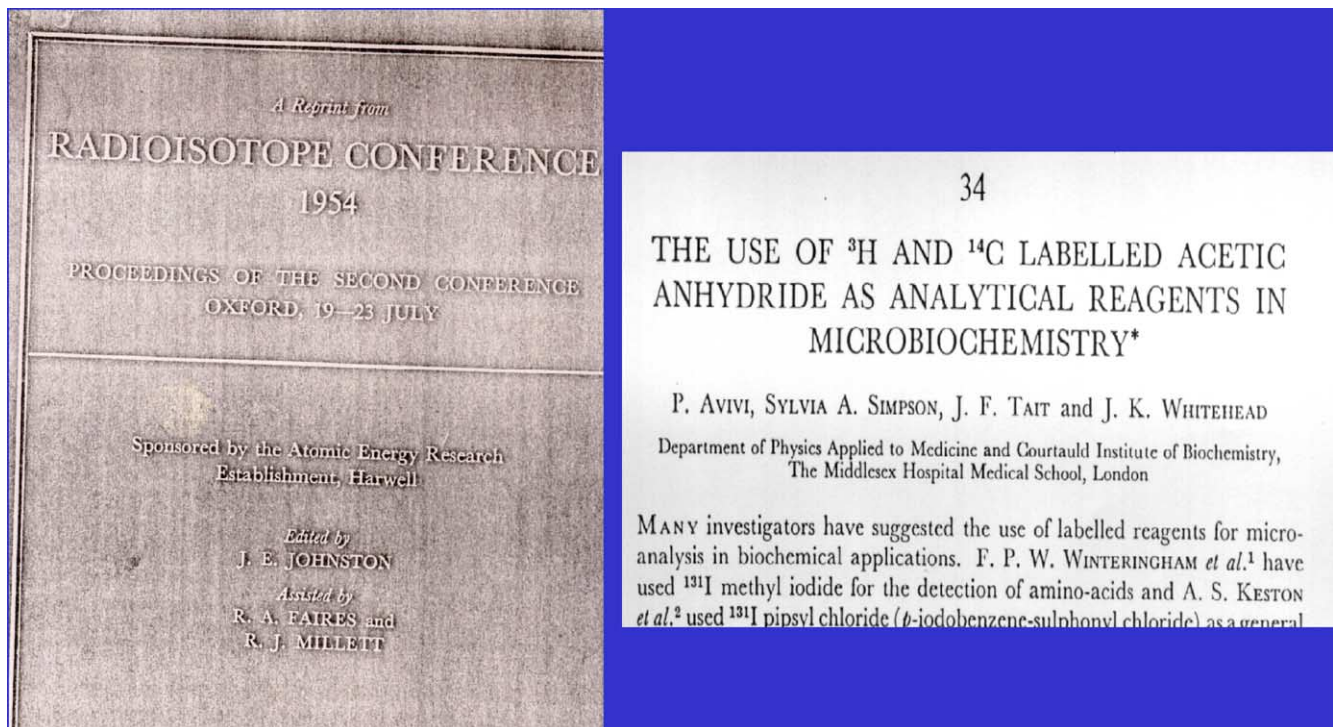| Species	Cortisol/corticosteroid
Rat (Nelson et al., 1950)	<50
Rabbit	<0.10
Ox (Hechter et al., 1951)	1
Ferret	1.5
Cat	4
Dog	5–6
Sheep	10–15
Monkey	20
Human (Peterson, 1959)	10

Fig. 19. Original publication using isotopic labelling as a measurement mode (from Avivi et al., 1954).

probably not as anti-phlogistic as cortisol. It is not clear why the higher order species should need protection for the heart. Could it be due to extra circulatory problems such as the vertical ambulatory position in these species? Clearly, the reason (if any) for the species difference in the pattern of adrenocortical secretion is still an intriguing problem in the adrenal.

13.3.2. The 1960–2003 research years

In the 40 years or so since the discovery years were behind us, much has been discovered but largely by creeping forward in many tiny steps. Over this period much of my own work (Coghlan) was done in the sheep with auto adrenal transplants as part of the Howard Florey Institute team.

Major contributions were made by many groups but in certain matters we had a major role, these are mentioned in the following section.

One of the first crucial observations was that the sensitivity of the response of the parotid gland to aldosterone was dependent on sodium status, negative sodium status markedly increasing the response (Blair-West et al., 1963).

A central issue was measurement. Isotope methods used during the identification were applied but the reagents were not 'hot' enough and the counters too insensitive (Avivi et al., 1954; Fig. 19). The advent of more sophisticated liquid scintillation counters made such methods more feasible (Kliman and Peterson, 1960). The availability of higher specific activity acetic anhydride allowed these methods to be pushed to their theoretical limit. As well ^{14}C labelled Aldosterone provided by NIH through Seymour Leiberman

and the Taits (Coghlan et al., 1966; Coghlan and Scoggins, 1967) probably only the Howard Florey Institute had these double isotope dilution assays set up as production lines. The focus on regulation/control of aldosterone secretion was impossible without measurements of the prevailing concentration of angiotensin II in peripheral blood. The demonstration by Goodfriend that antibodies to angiotensin could be produced, opened the way for effective radioimmuno assays sensitive enough for peripheral blood (Catt et al., 1967). Studies of the dynamics of angiotensin II production demonstrated that most angoitensin II was produced as it crossed peripheral circulatory beds (Fei et al., 1980). Lateralisation procedures for locating Conn's tumours were introduced and are still being used (Scoggins et al., 1972; Ma et al., 1986). The regulation of Aldosterone secretion was clearly multifactorial but the manner that the proximate stimuli interacted under different physiological circumstances has not been resolved. The gland is exquisitely sensitive to changes in plasma K (Funder et al., 1969) and shows changes in sensitivity to angiotensin II with changes in sodium status (Blair-West et al., 1973). Chopped adrenal tissue had a vogue and then isolated cells on which Sylvia and Jim spent so much energy, signal transduction, intracellular cascades, and protein induction. Now Eplerenone!

14. Conclusions

The generous support we have received during our scientific careers is acknowledged in the appropriate publications.

Recently, during our 'retirement', the support of the Royal Society Relief Fund has been essential for us to continue scientific work. The Welcome Museum of the History of Science has kindly stored our original correspondence with Professor Tadeus Reichstein during our collaboration (Tait and Tait, 1998b).

In conclusion, as this is the 50th aldosterone anniversary meeting, there are only a few of the pioneering workers, who could attend this conference and some of us who attended only just made it. We would like to pay a tribute to these former colleagues, too numerous to list here individually. Some of them have been mentioned in this talk; all of them have been dealt with inadequately because of time considerations. On the whole, the aldosterone field has been a pleasant area to work in and many of our rivals were also friends. It goes without saying that the recent clinical events in the field have delighted us, after, as one Searle senior scientist put it, 'aldosterone had disappeared from the map 15 years ago'. One cannot help wondering whether the physiological function of the hormone had also disappeared. In any case, it now seems certain that, because of the important basic research and the recent clinical applications, the field will continue to be in its present active state for a considerable time.

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