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18-HYDROXYCORTICOSTERONE: A REVIEW

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INTRODUCTION

Those corticosteroids (C21) in which the angular methyl group at C18 is oxidised either to a primary alcohol or to an aldehyde group possess unique structural features elicited by the close proximity of hydroxyl and carbonyl functions. Theoretically, a variety of structures may occur as a consequence of spontaneous formation of cyclic ketals or hemiketals which have a lower energy level than their open ring isomers. The number of possible structures increases with the number of oxidised functions. A high proportion of these compounds are relatively labile and precise characterisation has proved extremely difficult. The situation is further complicated by interconversion in storage where solvents, alcohols and traces of acid often induce spontaneous and reversible changes, forming a mixture of compounds whose physical properties differ sufficiently to allow chromatographic separation.

There are many reasons, apart from intellectual curiosity, why a solution to these problems must be found. From the standpoint of this review, the possibly important niche occupied by 18-hydroxycorticosterone in corticosteroid biosynthesis provides the most important.

Although it is a C18 alcohol, intermediate in structure between corticosterone (18-methyl) and aldosterone (18-aldehyde), it is a poor precursor in *in vitro* incubation systems for the major mineralocorticoid aldosterone and its biological properties and the control of its secretion also appear to differ from those of aldosterone. A knowledge of its chemistry and pharmacology is a necessary prerequisite to an understanding of its physiological and biochemical role.

HISTORY AND OCCURRENCE

A number of compounds have been isolated in which the 18-methyl group has been oxidised to a primary alcohol. In addition to 18-hydroxycorticosterone, the most important of these would seem to be 18-hydroxy-11-deoxycorticosterone (18-hydroxy DOC) and 18-hydroxyprogesterone [1]. Each compound will also give rise to a series of metabolites, excreted in the urine, which are the products of reduction and conjugation, usually with glucuronic acid.

In 1957, Ulick and Lieberman[2] published an account of analyses of urine from humans suffering from oedema probably resulting from aldosterone-induced sodium retention. From this source they isolated a compound, the most important features of which were the presence of an α-ketol function and the ability to form a triacetate. When this substance was oxidised with periodic acid, a lactone was formed which could then only produce a monoacetate. A formula of a $C_{21}O_5$ pregnane was calculated and the compound was inferred by the authors to possess hydroxyl groups at carbons, 3, 18 and 21 and carbonyl groups at 20 and possibly also 11. When radioactively labelled aldosterone was administered to the subjects, the compound was found to be labelled, suggesting that a new metabolite of aldosterone had been found. However, in further studies [3] the 'compound' was shown to be a mixture of tetrahydroaldosterone, the metabolite of aldosterone which was the labelled component, and 18-hydroxy-11-dehydro-tetrahydrocorticosterone, a metabolite, not of aldosterone but of 18-hydroxycorticosterone. In a footnote to this paper, the authors report unpublished studies by Llaurado which show that 18-hydroxycorticosterone has some mineralocorticoid activity, but is less potent than DOC in the adrenalectomised rat. By that time the parent compound, 18-hydroxycorticosterone had already been isolated from the adrenal tissue of the bullfrog [4] and the corresponding lactone from hog adrenals [147]. A summary of these early studies is available [5].

Ward and Birmingham[6, 7] and Péron[8–10] independently studying 18-hydroxy DOC, the major 18-hydroxy-corticosteroid in the rat, examined the properties of 18-hydroxycorticosterone as a subsidiary study in heterogenous material occupying the origin of their paper chromatograms. It was found to yield a diacetate on acetylation [6], to absorb U.V. light [6, 9], to reduce tetrazolium salts, albeit much more slowly than other corticosteroids and to fluoresce in alkaline solution [9]. The behaviour of the unknown compound and of authentic 18-hydroxycorticosterone were chromatographically indistinguishable in their free state, when acetylated and after oxidation with periodic acid [9]. 18-hydroxycorticosterone was eventually characterised from mouse adrenals by Raman, Ertel and Ungar[11].

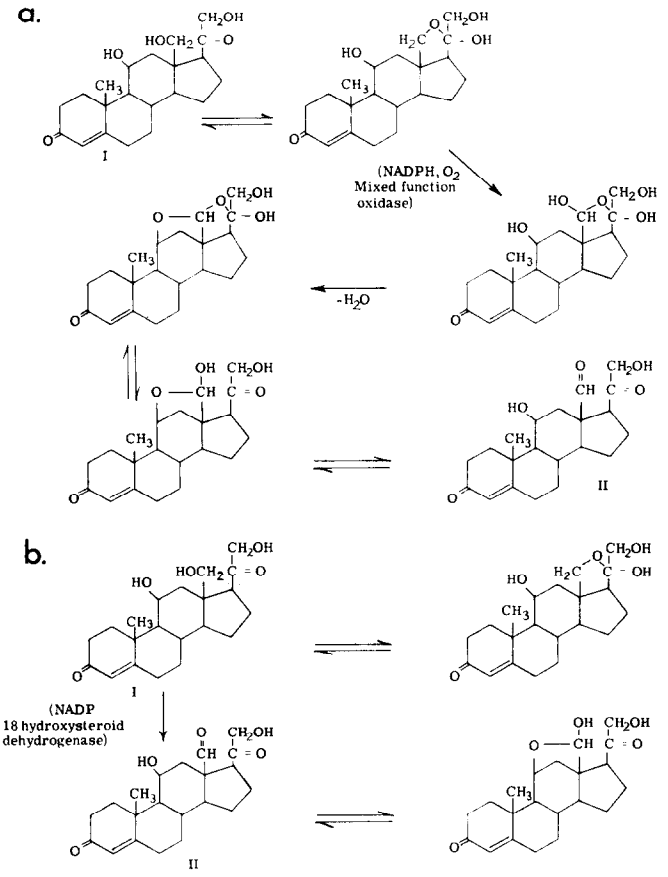

Fig. 1. Possible biosynthetic pathways for the conversion of 18-hydroxycorticosterone (I) to aldosterone (II).

Since that time the presence of 18-hydroxycorticosterone has been demonstrated in many other species. For example, it was shown to be released by incubated slices of bovine adrenal tissue [12] and it was also present in perfusates of whole glands from the same species [13]. In the first extensive account devoted to 18-hydroxycorticosterone, Sandor and Lanthier[12] give a detailed description of its chromatographic properties, derivative formation, its poor conversion to aldosterone *in vitro* and the quantitative importance of low polarity "tautomers". They also studied human adrenal tissue. In bovine tissue they were able to demonstrate the conversion of corticosterone to the 18-hydroxylated compound only in the zona glomerulosa, whereas Mulrow and Marusic[14], using homogenates of the same tissue, found the appropriate hydroxylase activity in the mitochondria of all three zones. The reason for this discrepancy is not clear. Although both pregnenolone and 11-deoxycorticosterone are converted to some extent to 18-hydroxycorticosterone by the rat adrenal fasciculata-reticularis, this conversion is much more efficient in the zona glomerulosa [15].

In addition to the rodents and the ox, 18-hydroxycorticosterone has recently been isolated from the adrenal glands of another mammal, the antarctic seal [16]. It was formed as a major product from radioactive pregnenolone or progesterone. No aldosterone was found in *in vitro* experiments in this species and 18-hydroxycorticosterone may be a major end product. The sheep also secretes 18-hydroxycorticosterone (17) and in man and the dog its plasma concentration is roughly equal to that of aldosterone [18, 19].

Lower down the evolutionary scale, the domestic duck adrenal secretes 18-hydroxycorticosterone in quantities approximating to those of corticosterone and more than twice as great as aldosterone [20] while in other birds it is quantitatively less important [21]. The compound is also secreted by reptiles [22]. The variable quantitative relationship from species to species between corticosterone and 18-hydroxycorticosterone on the one hand and 18-hydroxycorticosterone and aldosterone on the other, is striking and may give some clue to the physiological function of 18-hydroxycorticosterone and its importance as a precursor of aldosterone in these species.

CHEMISTRY

As already stated in the Introduction, 18-hydroxycorticosterone may exist either in open or cyclic forms (Fig. 1) and these probably coexist in equilibrium. For the open form, the side-chain could theoretically be

α or β orientated (i.e. 17α-pseudoequatorial; 17β-pseudoaxial). However, naturally occurring steroids invariably possess a β side chain, an α orientated side chain only being present in specifically prepared synthetic compounds [145, 146]. Only the 17β form could possibly give rise to the more stable hemiketal forms. These could also exist as two isomers in which the 20 → 18 hemiketal could possess either a 20S or 20R hydroxyl group. Investigation of these varied structures has been approached by many techniques and much of the information derived from studies of related compounds, particularly 18-hydroxy DOC, may also be relevant. For convenience, the results have been divided on the basis of the type of method used.

(a) *Dynamic methods*

Dynamic methods are destructive and include analytical chemical techniques, analysis of breakdown products obtained at high temperatures and analysis of high molecular weight derivatives by gas–liquid chromatography (glc) possibly linked to mass spectrometry (GCMS). Some examples are given below. The formation of an 18, 21 diacetate [23] or a dimethoxime, tri-trimethylsilylether derivative [24] suggests that at least part of the steroid is in the open form. Similarly, since only the open form can carry out reduction reactions and the formation of formazans with blue tetrazolium [9, 23], albeit much more slowly than other corticosteroids such as DOC or corticosterone [8, 9, 23], probably not all the steroid is in the hemiketal form. However, the comparatively poor reducing capacity of the compound may suggest that the equilibrium is much in favour of the hemiketal. The slow rate of reduction compared with other corticosteroids may reflect the slowness of conversion of the hemiketal to the open form. Formation of derivatives such as the diacetate and reduction reactions probably lead to replenishment of the open form by slow opening of the ring of the hemiketal component to maintain equilibrium. Thus, stoichiometric yields do not reflect the initial proportions of tautomers present in the equilibrium. While such methods may reveal the presence of an equilibrium, they yield no reliable quantitative information.

More direct evidence for the coexistence of two tautomers has been obtained by Prost and Maume[24] using GCMS. They treated the 18-hydroxy derivatives of DOC, corticosterone and progesterone with methoxyamine and with deuterated silylating reagents and obtained for each steroid three derivatives which had different glc retention times. For 18-hydroxycorticosterone, mass spectrometry showed that two of these obviously derive from the hemiketal form and the third from the open form. The proportion of the latter increased at the expense of the first two when the reaction was carried out at higher temperature or for a longer time, indicating a shift in the equilibrium in favour of the open form. The work of Génard and his colleagues [25] provides useful reference mass spectra for 18-hydroxy DOC, aldosterone and their respective γ lactones.

(b) *Static methods*

Techniques such as UV, infra red (IR) and nuclear magnetic resonance (NMR) spectroscopy do not involve the destruction of the compound under investigation and have been collectively termed static methods. Being non-destructive, such techniques are less liable to disturb the state of equilibrium and thus useful quantitative data can be obtained. For example, IR studies of 18-hydroxycorticosterone [23] and 18-hydroxy DOC [7, 9, 26] failed to demonstrate a pronounced absorption peak at 1700 cm^{-1} characteristic of compounds with unconjugated carbonyl groups. Such a group exists at C20 in the open form of 18-hydroxycorticosteroids. However, one of these studies [7] showed absorption at this wavelength 'that was sometimes barely evidenced as a shoulder'. Thus, the open isomer is present only as a minor component at equilibrium. Birmingham, Traikov and Ward[27] also used short wavelength ('near') IR spectroscopy, employing conventional spectrophotometers to examine over 30 steroids including 18-hydroxy DOC and 18-hydroxy-11-dehydrocorticosterone. An important finding was a shift of the hydroxyl absorption based in the 18-hydroxylated steroids which the authors ascribe to the attachment of the hydroxyl groups to carbons involved in a C—O—C linkage (see Fig. 1). Surprisingly, this interesting and simple method has not been further exploited.

N.M.R. studies of 18-hydroxy-DOC [28] seem to confirm the results of IR spectroscopy. Only the carbonyl at C3 could be detected and no signal corresponding to the carbonyl in the α ketol side-chain was evident.

Early studies (see [23]) led to the conclusion that, in contrast to steroids with 18-methyl groups, the open 17α forms of the 18-oxygenated steroids, only available through chemical synthesis, were more stable than their naturally occurring 17β isomers. However, there is as yet no evidence of the natural occurrence of the α orientated side-chain whereas, since only the 17β isomer can cyclise spontaneously, at least some of the open structure must exist in this form (see above). Moreover, only the 17β form could give rise to the 20 → 18 γ lactone upon periodate oxidation. There seems little doubt that the major component of the mixture is the thermodynamically more stable hemiketal tautomer of the 17β isomer. The relative abundance of the 20S and 20R stereoisomers of the hemiketal is not known.

Formation of artifacts in storage

It is an unfortunate characteristic of 18-hydroxylated steroids that in solutions in organic solvents, they form spontaneously and reversibly compounds of lower polarity. This tendency is enhanced by traces of acid in the solution and has been demonstrated for 18-hydroxyprogesterone [29], 18-hydroxy DOC

[8, 13, 30] and 18-hydroxycorticosterone [14, 31]. As solvents, methanol and ethanol are particularly troublesome in this way but acetone and, to a minor extent, chloroform, also allow formation of artifacts. An early attempt to examine this problem is described by Dominguez[30] using 18-hydroxy DOC. He distinguishes two forms, a less polar (L) and a more polar (M) form and states the following possible structures:

(i) in wet solvents, both open and 20 → 18 hemiketal forms may occur

(ii) two diastereoisomers of the 20 → 18 hemiketal form may occur,

(iii) the L form could be a dimer in equilibrium with its constituent monomer, the M form

(iv) the L form may be a product of reaction with the solvent, probably an alkyl ketal.

The very small proportion of the open form makes (i) untenable and Dominguez[30] points out that the polarity difference between the L and M forms is probably too great to be explained by (ii). Thus only (iii) and (iv) remain to be considered. The early work of Dominguez[30] has acted as a basis for more recent studies with improved analytical techniques.

Dimerization

18-Hydroxycorticosterone formed from corticosterone by microbiological hydroxylation has been isolated from the culture media as a dimer which, it was suggested, consisted of two monomers, linked together at their C20 and C21 loci [32]. NMR evidence for dimerization of 18-hydroxy DOC in methanol and dimethyl sulphoxide is provided by the work of Génard *et al.*[28]. Using a rather different approach, Damasco and Lantos[33] used Sephadex LH20 under conditions in which it behaved as a lipophylic mesh from which a variety of monomeric steroids were eluted with identical retention volumes. Under these conditions, the M form of 18-hydroxycorticosterone eluted with the monomeric compounds whereas the L form had a much lower retention volume. An isoprenol with a molecular weight of approximately 1000 eluted ahead of the L form. This indicates that the molecular weight of the L form is considerably higher than that of the M form and is consistent with dimer formation. The structure of the dimer remains a subject of discussion.

The hydroxyl at C20 of the hemiketal is likely to be labile because of its proximity to the oxygen bridge. It is therefore reasonable to infer that different dimerization reactions have in common the lowering of the dipole moment at C20. Dimerization may result from the substitution of the hydroxylic proton by a whole monomeric unit covalently linked at C20. Alternatively, the second monomer may be linked to an oxygen function by hydrogen bonds. In either case, further stabilisation may be provided by additional links at C18 or C21. A third possible substitution is by an alkyl group (see below).

Formation of alkyl-ketal derivatives

Traces of acid in alcoholic solutions of 18-hydroxysteroids increase the proportion of the L form and for this reason a small quantity of the base triethylamine is usually added [34, 35]. Génard *et al.*[28] in their NMR study quoted above found C20 methoxy derivatives of 18-hydroxy DOC in wet methanol solutions. However, perhaps the most convincing demonstration of the presence of non-polar alkyl derivatives is that of Roy, Ramirez and Ulick[36]. On acetylating 18-hydroxy DOC from an ethanolic solution which had been left to stand, they were able to characterise by means of mass spectroscopy two principle products, 18-hydroxy DOC 21 monoacetate and a more abundant, less polar acetate, 20 ethyl ketal 21 monoacetate, indicating that the parent compounds were 18-hydroxy DOC and its 20-ethoxy derivative. The corresponding 20-methoxy compound was found in methanol solutions. Alcohols of all grades reacted in this way and the reaction was faster in anhydrous solvent and in more dilute solutions. The reaction depended on catalytic traces of acid and could be prevented by redistilling the solvents from alkali or by adding triethylamine.

From the foregoing discussion it is evident that there must be more than one L form (for further discussion see [37] and addendum to this review). On paper chromatography the most commonly found L form migrates approximately four times as far as the M form but another component is frequently found which is even less polar [37]. It should now be possible to avoid the formation of artifacts in solution but much remains to be discovered of the state of 18-hydroxycorticosterone in biological tissues and the significance of the different forms in corticosteroid biosynthesis.

BIOCHEMISTRY

Since corticosterone is readily converted to aldosterone it is likely to lie on the main biosynthetic route to the mineralocorticoid (12, 38–42). 18-Hydroxycorticosterone is an obvious candidate as an intermediate compound in this pathway. Theoretically, the conversion of corticosterone to aldosterone (i.e. 18-methyl to 18-aldehyde) could occur in two steps, an 18-hydroxylase-catalysed formation of 18-hydroxycorticosterone followed by an 18-hydroxysteroid dehydrogenase-controlled oxidation to aldosterone. The first of these steps has been studied extensively. However, considerable ambiguity has arisen from the use, in some publications, of the term '18-hydroxylase' to denote the enzyme control of the complete conversion of corticosterone to aldosterone while others more properly restrict its use to the conversion of the angular methyl to a primary alcohol group. To some extent this confusion is due to a lack of knowledge of many aspects of the molecular mechanisms involved in the two stage reaction. For a review of the

earlier literature covering the biochemistry of 18-hydroxy-corticosterone, the reader is referred to Rosenthal and Narashimhulu[43] and Müller[44].

The importance of 18-hydroxycorticosterone as a precursor of aldosterone has by no means been definitely established and evidence is often conflicting. Indirect evidence based on experiments in which metabolites were 'trapped' by dilution with radio inert steroid [45] suggests that conversion is possible. However, as a precursor, corticosterone has been found to be much more effective than 18-hydroxycorticosterone *in vitro* by a factor of between 1.5 [46] and 80 [44]. Whitehouse and Vinson[47] found that both 11-deoxycorticosterone (DOC) and corticosterone were more efficiently converted to aldosterone than the 18-hydroxylated compound by rat adrenal cortex. Although Pasqualini[48] demonstrated that 10% of added 18-hydroxycorticosterone was converted to aldosterone in the presence of NAD and fumarate by human adrenal tissue, no comparison was made with the use of other precursors such as corticosterone. Moreover, although the conversion of the 18-hydroxysteroid to an aldehyde might be expected, as postulated above, to be catalysed by a hydroxysteroid dehydrogenase, the requirement of reduced NADP and the components of the cytochrome P450 system noted in most [46, 49], but not all, experiments is more in keeping with a steroid hydroxylase enzyme system. The dilemma resolves into two main components:

(1) Is 18-hydroxycorticosterone an important precursor of aldosterone *in vivo* and, if so, why is it so poorly converted *in vitro*?

(2) Are there one or several enzymes involved in the conversion and, if present, what is the role of the hydroxylase?

There follows a consideration of the nature of the immediate precursor of aldosterone and the enzymes regulating its conversion.

Zonation

Although, in bovine adrenal homogenates at least, 18-hydroxycorticosterone is synthesised in each zone of the adrenal cortex [14], nevertheless it appears to be mainly synthesised in the zona glomerulosa [50] and aldosterone, so far as is known, is the only corticosteroid whose synthesis is confined to this zone [51]. The other important 18-hydroxycorticosteroid is 18-hydroxy DOC which is derived mainly from the zona fasciculata-reticularis [50, 52]. It is not known whether the 18-hydroxylation of DOC and corticosterone in this zone is catalysed by the same enzyme.

Enzyme location and co-factor requirements

Psychoyos, Tallan and Greengard[53] found that homogenates of the adrenal glands of the American bullfrog, a species in which aldosterone is the principal corticosteroid secreted [4, 51, 54], were a particularly good source of enzymes for both aldosterone and 18-hydroxycorticosterone synthesis. In both cases, activity was located in the mitochondrial fraction but there were interesting discrepancies between the steroids on the effect of the soluble and microsomal fractions on the rate of synthesis. Mitochondria alone were capable of synthesising as much 18-hydroxycorticosterone from labelled corticosterone as the complete homogenate although addition of the soluble and microsomal fractions, together but not separately, gave some further improvement in steroid production. In contrast, mitochondria produced less than half the quantity of aldosterone synthesised by the complete homogenate. The addition of the soluble fraction to the mitochondria considerably enhanced the conversion of corticosterone to aldosterone while the low-speed sediment was less effective. A possible interpretation of these results is that the '18-hydroxylase' resides in the mitochondria, deriving its co-factors also from this source. The final oxidation step must require non-mitochondrial factors.

Aldosterone and 18-hydroxycorticosterone synthesis by adrenal mitochondria of the bullfrog [53, 55] sheep [46] or rat [49] requires the presence of NADPH. This may be provided by direct addition [46, 49], by a classical generating system [49] or by the addition of NADP and a citric acid cycle intermediate [53, 55]. Malate is more effective than fumarate [55]. A fumarase has been identified as the factor present in the soluble fraction necessary for mitochondrial aldosterone synthesis [55] presumably indicating that NADPH is necessary. The effect on aldosterone synthesis of using NAD or NADP instead of their reduction products is another subject of controversy. In bovine adrenal homogenates, Kahnt and Neher[56] showed that both corticosterone and 18-hydroxycorticosterone were converted to aldosterone at similar high rates using NAD, NADP or NADPH but this conflicts with experiments of Raman *et al.*[46] using ovine adrenal homogenates, who found that the transformation of 18-hydroxycorticosterone to aldosterone required added or generated NADPH. NADP or NAD alone led to the oxidation of the 11-hydroxyl group. The product, 18-hydroxy-11-dehydrocorticosterone, has chromatographic properties similar to aldosterone and this may be an explanation of the disagreement. In a recent study, Aupetit *et al.*[151] showed that 'endogenous' 18-hydroxycorticosterone was less effectively converted to aldosterone than synthetic 18-hydroxycorticosterone but that the 'endogenous' material becomes more effective when isolated. They conclude that the 18-hydroxysteroid must be retained in an active, enzyme-bound state in transit from corticosterone to aldosterone and that, once released, it is no longer an effective precursor. However, once isolated it may undergo physico-chemical changes which restore its activity as a precursor.

Effect of cation concentrations

Sodium depletion or potassium loading stimulate the secretion of aldosterone and therefore directly, or

indirectly, affect its biosynthesis. Since Rosenfeld, Rosemberg, Ungar and Dorfman[57], shortly after the discovery of aldosterone, found that alterations of Na/K balance induced an increase in the secretion of biologically active material by perfused calf adrenal glands, there have been many studies of the effects of these cations but as yet information is conflicting and any conclusions must remain tentative.

Research in this field can be divided roughly into three categories.

(a) Changes in cation concentration in incubation, superfusion or perfusion systems of isolated adrenals or adrenal tissue (57–66).

(b) Local manipulation of adrenal artery cation concentration with the adrenal gland *in situ*. This approach has been reviewed by Blair-West *et al.*[67].

(c) Dietary or physiological manipulation of sodium and potassium status [61, 68].

It is generally accepted that a decrease in Na/K ratio stimulates aldosterone biosynthesis [57–63] and secretion [61, 67, 68] and that *in vitro*, this stimulation can be largely attributed to the increasing potassium component of the ratio with a maximum effect at approximately 8 mEq/l [69, 70]. This is to some extent corroborated by *in vivo* experiments in dogs in whom sodium intake was maintained constant and potassium intake varied to give a range of plasma potassium concentrations [71]. Maximum stimulation of plasma aldosterone concentration occurred at approximately 8 mEq/l. The addition of potassium ions together with a withdrawal of sodium ions appears to affect aldosterone biosynthesis at two loci, at a step before corticosterone synthesis and in the conversion of corticosterone to aldosterone [44, 68]. Conversion of corticosterone to aldosterone is enhanced by moderate sodium depletion in the sheep adrenal gland *in situ* [72]. Changes in intracellular potassium levels may not be the cause of altered biosynthesis since these do not correlate well with steroid production rates *in vitro* [73, 74]. The precise biochemical mechanism remains obscure largely because of the difficulty in reproducing the effects of electrolyte status in cell free systems. For example, Marusic and Mulrow[61] have clearly demonstrated that adrenal mitochondria from sodium-deprived rats showed a 200% increase in the conversion of corticosterone to aldosterone *in vitro* compared with those from rats on a normal sodium intake. However, they failed to alter the yield of aldosterone from mitochondria by altering the sodium or potassium content of the incubation medium whether the mitochondria came from normal or sodium depleted rats.

Other cations may be involved in the control of aldosterone biosynthesis. For example, ammonium ions stimulate aldosterone biosynthesis but their effect does not appear to be on the conversion of corticosterone to aldosterone [44]. On the other hand, the divalent magnesium ion is necessary for the synthesis of both 18-hydroxycorticosterone and aldosterone by adrenal mitochondria and, while magnesium can be replaced effectively by manganese for aldosterone biosynthesis, the latter is only partially effective in maintaining 18-hydroxycorticosterone production [53]. Conversely calcium ions can replace magnesium for 18-hydroxycorticosterone but not for aldosterone synthesis.

ACTH and angiotensin

The effects of ACTH and angiotensin II on corticosteroid biosynthesis have received much attention in recent years and the reader is referred to a number of excellent reviews dealing with this subject [1, 44, 75–78]. However, while the rate of aldosterone synthesis in response to these stimuli has been studied carefully, the specific role of 18-hydroxycorticosterone, both its availability and rate of oxidation to aldosterone, remains unresolved.

For ACTH, the situation may be summarised as follows. The peptide appears to act at a number of sites in the biosynthetic pathway [79–84], but its major effects probably occur early in the biosynthetic sequence, altering the rate of hydrolysis of cholesterol esters, the availability of cholesterol within the mitochondrion and the rate of desmolase-catalysed side chain cleavage [75]. There is recent evidence that ACTH increases the concentration of adrenodoxin, the adrenocortical non-haem iron protein important in steroid hydroxylase activity [85], and this may explain the more general effects of ACTH such as on 11β-hydroxylase activity. In the rat ACTH inhibits the formation of aldosterone from corticosterone [81, 83, 90]. The major action of the hormone depends on cyclic AMP formation [86] and reciprocal cyclic AMP and cyclic GMP changes may occur at different levels of ACTH stimulation [87]. ACTH increases aldosterone production *in vitro* and in this it may be replaced by cyclic AMP [88]. However, the rate of 18-hydroxylation of corticosterone or formation of aldosterone from corticosterone is unaffected [89], or as mentioned earlier, slightly impaired at least in the rat [81, 83, 84, 90]. Boyd, Page and Mulrow[91] found conversion of corticosterone to aldosterone to be enhanced in tissue from sodium depleted rats but hypophysectomy of the donor animals, while reducing the rate of aldosterone synthesis, failed to alter the efficiency of conversion. In addition to its activation of the 'second messenger' system, it may also act by changing the physical state of these precursors. Vinson and Whitehouse[92] in a series of dialysis experiments, showed that ACTH *in vitro* may release precursors such as DOC from a protein-bound to a free state. Whether this also occurs *in vivo* remains to be established.

Angiotensin II is preferentially bound in adrenal tissue [93] and increases aldosterone production *in vitro* in zona glomerulosa cells [62, 63, 70A, 94] at concentrations within the physiological range. It is a more potent stimulus to aldosterone production *in vitro* than ACTH [95]. However, it also markedly stimulates corticosterone synthesis, again indicating

a major influence early in biosynthetic pathway [63, 96, 97]. *In vitro*, its action is not restricted to the zona glomerulosa [98], but the glomerulosa is the most sensitive region of the adrenal cortex [94].

This early effect may not be identical with that of ACTH. If plasma concentrations of corticosteroids can be accepted as a rough index of biosynthetic activity in man, recent studies on the interaction of ACTH and angiotensin II in normal human subjects may provide preliminary evidence of this. In man at least, angiotensin II appears to inhibit ACTH release [99] although this is disputed [100]. When angiotensin II is infused alone only 18-hydroxycorticosterone and aldosterone levels correlate with concurrent plasma angiotensin II concentrations [101] but if the infusion is carried out in the presence of a low constant rate infusion of ACTH, positive correlation of plasma angiotensin II with DOC, corticosterone, 18-hydroxy DOC and cortisol is also obtained [99]. That the presence of ACTH is necessary for the earlier action of angiotensin II may be interpreted as indicating that the octapeptide affects a later locus than ACTH. This may be at the stage of the 3β-ol dehydrogenase, 5-4-ene isomerase catalysed reactions [102].

Again, the effect of angiotensin II on 18-hydroxycorticosterone metabolism in the adrenal has received little attention, but evidence of a later effect on biosynthesis in rat adrenal tissue is provided by the work of Marusic and her colleagues [103, 104]. They report that the octapeptide increases the conversion of corticosterone to aldosterone and that this effect is enhanced if tissue from sodium depleted rats is used. More recently interest has been shown in the role of the heptapeptide des-asp^1 angiotensin II (angiotensin III) in the control of aldosterone production. While it has been shown to be equipotent with the octapeptide in raising plasma aldosterone levels in the rat [105, 106], sheep [107] and man [108], it is not known whether its mechanism of action is identical to that of angiotensin II or indeed whether the octapeptide is converted to the heptapeptide within the adrenal cortex. Its action may not depend on cyclic AMP formation, but the involvement of other cyclic mononucleotides cannot be excluded [109].

Inhibiting factor

Psychoyos *et al.* [53] showed that homogenates of bovine or rat adrenal glands caused a 75% inhibition of 18-hydroxycorticosterone and of aldosterone synthesis by bullfrog adrenal homogenates. The inhibitory factor from the bovine adrenal preparation could be destroyed by heating to 100°C. Corticosteroid biosynthesis can also be inhibited by synthetic substituted pyridine derivatives. Comparative studies of a large series of these are the subject of a series of papers by Kahnt and Neher[148–150]. A number of them are thought to competitively inhibit steroid–cytochrome P450 binding, thus affecting hydroxylase activity (see below). 4-Monosubstituted derivatives are more effective inhibitors than 3-monosubstituted compounds when 18-hydroxylation is compared with 17-hydroxylation [149].

Nature of enzymes and precursors

The term "mixed function oxidases" was first used by Mason[110] to describe enzymes which catalyse the introduction into a substrate of an atom of molecular oxygen coupled with the oxidation of a pair of electrons from a specific donor.

$$\text{i.e. } AH + 2e + O_2 \rightarrow A\,OH + O^{2-}$$

Steroid hydroxylases are mixed function oxidases whose electron donor is NADPH or less commonly NADH.

$$\text{i.e. } NADPH + H^+ + \text{Steroid } H + O_2 \rightarrow NADP^+ + \text{Steroid } OH + H_2O$$

Since a knowledge of this reaction mechanism is fundamental to an understanding of 18-hydroxylation and dehydrogenation, it is here discussed in some detail. More general reviews have appeared recently [77, 111]. Omura, Sato and Cooper[112] and Estabrook, Cooper and Rosenthal[113] were responsible for suggesting that microsomal mixed function oxidases required a CO-binding pigment for oxygen activation (see also [114]). CO inhibited 21-hydroxylation of 17α-hydroxyprogesterone in the synthesis of 11-deoxycortisol but the inhibition was effectively reversed by light at a wavelength of 450 nM. The photometric difference spectrum (the CO–pigment complex corrected for the absorption of the pigment itself, the pigment in both cases being in the reduced state) also showed a maximum at 450 nM. Addition of a steroid substrate, 17α-hydroxyprogesterone, to microsomes resulted in rates of NADPH oxidation and deoxycortisol formation which were equivalent.

Cytochrome P450 also occurs in the mitochondria [115, 116] supplementing the respiratory chain. Adrenal mitochondrial steroid hydroxylases such as the 11β-hydroxylase which catalyses the conversion of DOC to corticosterone and the 18-hydroxylase acting on corticosterone, depend for their activity on the presence of cytochrome P450.

In view of these findings and also the observation that NADPH was required for aldosterone biosynthesis, Greengard, Psychoyos, Tallan, Cooper and Rosenthal[117] tested the possibility that a 'mixed function oxidase' using cytochrome P450 was involved in the two stage reaction. Using the criteria noted above [113] they were successful in demonstrating such a participation in the conversion of corticosterone to aldosterone by frog adrenal mitochondria. From these results they postulated that the hydroxylation step which occurs in the formation of aldosterone produces 18-hydroxycorticosterone and they assumed the final step to be catalysed by an 18-hydroxysteroid dehydrogenase. However, from more recent studies using dog adrenal mitochondria,

Marusic and Mulrow[61] have concluded that the second stage reaction, the oxidation of the 18-hydroxy group, may also be controlled by a 'mixed function oxidase'. Only aerobic conditions gave reasonable yields of aldosterone from either corticosterone or its 18-hydroxy-derivative indicating the need for molecular oxygen [49]. NADPH was without effect.

The questions of which enzyme(s) catalyse the oxidation of 18-hydroxycorticosterone to aldosterone and what is the nature of the immediate aldosterone precursor are inextricably linked. The poor precursor capacity of 18-hydroxycorticosterone has previously been remarked upon and one possible explanation of this can be found in its hemiketal structure [41]. This structure, lacking a C18 hydroxyl, does not favour the dehydrogenase-dependent conversion to aldosterone. However, it could explain the need for a 'second hydroxylase' [49] to catalyse the introduction of an additional hydroxyl at C18 and facilitate the formation of an oxygen bridge between this carbon and C11 (see Fig. 1(a)). There follows a series of molecular rearrangements (see Fig. 1(a)). This contrasts with the more direct pathway in which an 18-hydroxysteroid dehydrogenase would catalyse the oxidation of 18-hydroxycorticosterone to aldosterone, both compounds remaining in the open form, and aldosterone would then assume its hemiketal form (Fig. 1(b)). In fact both pathways may coexist in the adrenal cortex and their activity in relation to each other may be modulated, for example, by the prevailing physiological conditions which might determine whether NADPH and 18-hydroxycorticosterone hemiketal were present, favouring pathway a (Fig. 1) or whether the corticosteroid was present in the open form and the cofactor in its unreduced state in which case pathway b (Fig. 1) might predominate. It should be emphasised that it has not yet been possible to stabilise the open form of the steroid triol. The finding that one of the less polar transitions of 18-hydroxycorticosterone (L form) is converted more readily than the more commonly isolated hydrophylic form (M form) [118] tends to complicate the problem. However, elucidation of the structures of the less polar compound and the compounds discussed by Aupetit *et al.*[151] might help to clarify our ideas on this controversial subject.

ASSESSMENT OF 18-HYDROXYCORTICOSTERONE SECRETION

Steroid production by the adrenal cortex can be monitored in the intact subject by analysis of urine for steroid metabolites or by measuring the peripheral plasma concentration of the hormone. Urine analysis may be used to determine either excretion or secretion rates. The former approach, more common in clinical practice, allows an integrated assessment of adrenocortical activity over the period of urine collection and avoids ambiguities produced by minute to minute fluctuations in secretion. However, short term changes are more relevant to studies of mechanisms of control of secretion and for this purpose analysis of peripheral plasma would seem to be the method of choice.

(a) *Urine analyses*

For 18-hydroxycorticosterone, the metabolite 18-hydroxytetrahydro-11-dehydrocorticosterone (18-hydroxy THA) glucuronide has been most frequently used as an index. For example, David, Golan and Drucker[119] used the Porter Silber reaction (see above) to measure 18-hydroxy THA excretion rate in two children with an inborn error of aldosterone biosynthesis. Unfortunately, they give no performance criteria and no normal excretion rate values, thus making evaluation of the method impossible. Shackleton, Honour, Dillan and Milla[120] identified 18-hydroxytetrahydrocorticosterone in addition to 18-hydroxy THA as a urinary metabolite of 18-hydroxycorticosterone. Identification by mass spectroscopy was tentative only. Excretion rates of the two compounds were measured by glc of their respective methyloxime-trimethyl silyl ethers and were roughly equal. The normal range for infants is given as 50–200 μg 24 h^{-1} which is higher than that of tetrahydroaldosterone.

The secretion rate of a hormone can be calculated by measuring the specific activity of a unique metabolite of 18-hydroxycorticosterone in urine following the injection of radioactively labelled hormone of known specific activity. Secretion rate is derived by the principle of dilution. Ulick, Nicolis and Vetter[5] used the original observation [3] that 18-hydroxy THA originated solely from 18-hydroxycorticosterone to devise a secretion rate method for use in man. [^{3}H]-Labelled 18-hydroxycorticosterone was obtained biosynthetically but is now available from commercial sources. The metabolite glucuronide from urine collected over a known period of time was hydrolysed and the free steroid purified chromatographically after oxidation to the γ lactone. Acetylation with [^{14}C]-acetic anhydride of known specific activity allowed steroid mass to be measured but the authors also suggest that colorimetry, such as the Porter Silber reaction (see above), may be used instead. Normal secretion rates varied from 135 to 460 μg 24 h^{-1}, again suggesting that more 18-hydroxycorticosterone than aldosterone is secreted. It is salutary to realise that, although this technology has been available for more than ten years, it has not been very fully exploited.

(b) *Plasma analyses*

Although adrenal vein 18-hydroxycorticosterone analyses have been published [121], direct adrenal vein sampling is likely to disturb severely the function of the gland and may thus have little practical application. One exception may be the techniques used for localising adrenal lesions in man which involve adrenal vein catheterisation. However, for the pur-

poses of investigating short-term changes requiring multiple blood sampling, peripheral plasma or blood samples—in normal man at least—are the only media available. 18-Hydroxycorticosterone is dilute in plasma and only recently have techniques of sufficient sensitivity become available for adaptation to this problem. So far only two techniques have been used, radioimmunoassay and glc with electron capture detection. In the first of these [122] antibodies were raised to the γ lactone of 18-hydroxycorticosterone thus avoiding the problems of isomerisation discussed above. The authors have also taken advantage of the unique situation in the domestic duck (see above) by using mitochondria from the adrenal cortex of this animal to convert [^{3}H]-corticosterone to [^{3}H]-18-hydroxycorticosterone. The antibody cross-reacted 100% with 18-hydroxy DOC γ lactone so that biological extracts were subjected to paper chromatography before radioimmunoassay. Normal human plasma concentrations by this method ranged from 6 to 16 ng $100\,ml^{-1}$ in recumbent subjects and 13 to 42 ng $100\,ml^{-1}$ when the subjects were standing. In a method using gas-liquid chromatography [123, 124] artifacts were again avoided by oxidising 18-hydroxycorticosterone, in this case to its 11 oxo γ lactone and purification involved a single paper chromatography stage. The derivative was then made sensitive to the electron capture detector by esterification with heptafluorobutyric anhydride to form the enyl ester. Normal plasma concentrations, again in man, ranged from 3 to 25 ng $100\,ml^{-1}$. The performance of the two methods as published is similar, radioimmunoassay being somewhat less sensitive but more precise.

FACTORS AFFECTING 18-HYDROXYCORTICOSTERONE SECRETION

The secretion of corticosteroid hormones is influenced by ACTH, released from the anterior pituitary in response to stress, by electrolyte status particular sodium and potassium levels, and by the renin-angiotensin system. The control system is further complicated by interaction of these individual factors, particularly between sodium status and angiotensin or ACTH, and also because individual steroid hormones are affected to varying extents. For example, ACTH has a greater effect on cortisol than on aldosterone secretion, whereas the reverse is true of changes in sodium balance. While no detailed studies of the control of 18-hydroxycorticosterone secretion were undertaken until recently, most aspects were studied briefly by Ulick and his co-workers [5] several years ago and recent studies tend to confirm the qualitative aspects of their results while modifying quantitative aspects. The general conclusion was that 18-hydroxycorticosterone secretion moved in close parallel to that of aldosterone in both healthy and diseased subjects. The ratio of the secretion rates of the two compounds remained constant with a correlation coefficient of 0.84 in normal subjects. To a large extent, changes in plasma concentration or secretion rate reflect the changes in biosynthesis discussed earlier in this review.

ACTH

The possible mechanisms of action of ACTH have been discussed. Administration of sufficient ACTH to give a 5-fold increase in cortisol secretion gave only a doubling of 18-hydroxycorticosterone secretion in normal human subjects while dexamethasone, which suppresses ACTH release, failed to decrease 18-hydroxycorticosterone secretion [5]. Although a case of Addison's disease had subnormal secretion rates of both aldosterone and 18-hydroxycorticosterone, absent ACTH secretion in a case of hypopituitarism was associated with normal levels of both hormones [5]. Similarly, David *et al.*[119] report the relative lack of effect of either dexamethasone or ACTH in changing 18-hydroxycorticosterone secretion in children with deficient conversion of the hydroxysteroid to aldosterone. However, more recent studies in normal human subjects show that plasma concentration follows a diurnal pattern similar to that of ACTH with values higher in the morning than the evening [124]. Moreover, in three normal subjects treated with dexamethasone, plasma concentrations of 7, 4 and 6 ng $100\,ml^{-1}$ increased to 46, 27 and 37 ng $100\,ml^{-1}$ respectively after 2.5 μg of $^{1-24}$ACTH had been infused during a period of one h [125]. Infusion of ACTH at higher, pharmacological doses resulted in further rises of plasma 18-hydroxycorticosterone concentration.

Further evidence for a marked and positive effect of ACTH can be obtained from a study of a subject with a defective 17α-hydroxylase system. Such subjects are incapable of synthesising cortisol and ACTH levels, unsuppressed by the negative feedback mechanism, are very high. In these circumstances, plasma 18-hydroxycorticosterone concentration has been reported to be high and to be reduced to normal by dexamethasone therapy [126]. In this single subject aldosterone concentration was low, presumably due to the excessive sodium retention caused by hypersecretion of ACTH-dependent mineralocorticoids such as DOC and corticosterone. In these circumstances, ACTH would seem to have been a more powerful influence over 18-hydroxycorticosterone secretion than sodium status, while the reverse was true of aldosterone.

In conclusion, there seems little doubt that ACTH is capable of stimulating 18-hydroxycorticosterone secretion although it may not be necessary for maintaining normal basal levels. The importance of the anterior pituitary compared with other influences in normal subjects is not known and the marked interaction of sodium status and ACTH, demonstrated in relation to aldosterone secretion [127, 128] has not been studied.

Electrolyte status and the renin-angiotensin system

Sodium depletion increases the secretion rate and plasma concentration of aldosterone while sodium loading has the reverse effect. This is in agreement with *in vitro* studies of biosynthesis and is at least partially due to increased conversion of corticosterone to aldosterone (see above). Although direct effects of sodium status on adrenal function in intact animals cannot be excluded, it is likely that changes of sodium status within the normal physiological range are translated for the adrenal in terms of the renin-angiotensin system. Angiotensin II infusion increases plasma aldosterone concentration in normal human subjects and the effect is greater in sodium deplete than in sodium replete subjects [129]. The mechanism of this apparent sensitization of aldosterone secretion to angiotensin II stimulation by sodium deprivation is not understood. This subject has been reviewed recently [130]. Changes of sodium status are inextricably associated with reciprocal changes in potassium levels and, in considering the results of experimental manipulation of sodium metabolism, the possibility that these are due to changes in potassium balance must at least be entertained. Again the early work of Ulick *et al.*[5] showed a three to five-fold rise in the secretion rates of aldosterone and 18-hydroxycorticosterone following six days of dietary sodium restriction and diuretic administration. Martin, Edwards, Biglieri, Vinson and Bartter[122] report a ten-fold increase in plasma 18-hydroxycorticosterone concentration in sodium depleted subjects but no information on the duration of sodium restriction is given. In a series of normal subjects depleted of sodium to a variable extent, Mason, Fraser, Morton, Semple and Wilson[131] found the response of plasma 18-hydroxycorticosterone to increase with the severity of sodium loss and, possibly more significantly, to be greater in proportion than the rise in plasma aldosterone levels. The difference in response between aldosterone and its precursor was particularly marked in the more severely depleted subjects. DOC-induced sodium loading causes 18-hydroxycorticosterone secretion rate to fall [119].

The effect of angiotensin infusion on 18-hydroxycorticosterone secretion was inconclusive but nephrectomy in a subject with unilateral renal artery disease, a manoeuvre which probably reduced renin and therefore angiotensin II levels in plasma, was followed by a fall in secretion [5]. In a series of experiments in normal sodium replete human subjects, infusion of angiotensin II at a series of rates caused a marked increase in plasma 18-hydroxycorticosterone concentration and its angiotensin II dose–response relationship was parallel to that of aldosterone. No changes could be detected in the circulating levels of other corticosteroids [132]. Angiotensin II infusion also increased plasma 18-hydroxycorticosterone concentration in sodium-deprived subjects but, unlike aldosterone, evidence of sensitisation to angiotensin II by sodium depletion was obtained in only one of six subjects [131]. Thus, since the basal levels of 18-hydroxycorticosterone were higher than those of aldosterone and the response to angiotensin II in the sodium deplete state smaller in proportion, the 18-hydroxycorticosterone:aldosterone ratio fell across infusion. The response to angiotensin II infusion was not secondary to ACTH release since dexamethasone treatment failed to alter it.

Some implications of the response to sodium balance and angiotensin II infusion

Two main questions regarding the control of aldosterone secretion remain to be answered. Firstly, at what loci in the biosynthetic pathway do sodium, potassium and angiotensin II act and, secondly, what is the biochemical explanation of the sensitisation phenomenon described above? The results of following and comparing the changes in the peripheral plasma concentrations of the individual corticosteroids may go some way to solve these problems only if these changes reflect intra-adrenal activity. With this proviso, it seems probable that angiotensin II has an important action at, or earlier in the biosynthetic sequence than 18-hydroxylation since infusion into sodium replete subjects causes a rise in 18-hydroxycorticosterone levels. The same must also be true of sodium depletion and the results may indicate that the effect of sodium loss is secondary to the rise in angiotensin II production which occurs in response to loss of sodium. Whether the polypeptide acts as early in the pathway as ACTH cannot be established with any certainty (see [131, 132]). However, as discussed earlier, there is good evidence that at least one of its loci of action must occur before 21-hydroxylation [99]. The increase in the 18-hydroxycorticosterone:aldosterone ratio during sodium depletion may indicate that the oxidation of the 18-hydroxy group is rate limiting. The fall in this ratio when angiotensin II is infused, possibly indicating increased conversion to aldosterone, suggests a second, later locus of action of the polypeptide. The increase in the relative quantities of 18-hydroxycorticosterone available for oxidation to aldosterone may also be the explanation of sensitisation to angiotensin II. Such conclusions must, of course, be regarded as highly speculative, based as they are on certain assumptions, not the least of which is that 18-hydroxycorticosterone is the main precursor of aldosterone. Insufficient data from this type of study are available for comparison to be made with the *in vitro* studies described earlier.

CLINICAL DATA

18-Hydroxycorticosterone analyses have not found general use in medicine but have been mainly restricted to studies of defects in aldosterone biosynthesis. Many of the reported cases have been listed and

briefly reviewed by Shackleton *et al.*[120]. A further case is described by Shackleton and Honour[152]. Such cases are characterised by the general symptoms of excessive sodium loss, remediable by salt administration and mineralocorticoid therapy [133, 134]. In these children, 18-hydroxycorticosterone secretion [5] or 18-hydroxy THA and 18-hydroxytetrahydrocorticosterone excretion (see [120]) are raised often to very high levels but fall to normal following therapy. Some details of these studies have already been given. In a recent article dealing with specific derangement of aldosterone biosynthesis, Ulick[135] states that the ratio of 18-hydroxycorticosterone to aldosterone remains fairly constant unless the corticosterone methyl oxidase enzyme system is affected. In this case, the ratio is increased.

In other cases, the production of 18-hydroxycorticosterone is high when that of aldosterone is high [5], in cases of secondary hyperaldosteronism such as cirrhosis with ascites and renal artery stenosis and in primary hyperaldosteronism [136].

BIOLOGY

Very little is known about the physiological function of 18-hydroxycorticosterone in vertebrates. Although in humans 18-hydroxycorticosterone secretion responds, in general, to the same stimuli as the secretion of aldosterone, there is no evidence that the two steroids have similar biological effects. Lanthier and Sandor[137] reported that 500 μg of 18-hydroxycorticosterone repeatedly infused into a duck increased the secretion volume of its salt-secreting gland and hence the gland's total sodium output. Similarly, recent experiments on adrenalectomized rats showed a significant rise in the urinary sodium output following the injection of 3 μg of the steroid, although higher doses produced a small but significant sodium retention [138]. Mention may also be made of the observations of Llaurado quoted earlier. In infants with defective aldosterone biosynthesis, characterised by an excessive sodium loss, 18 hydroxycorticosterone secretion, or the excretion of its urinary metabolites, may be considerably increased and it is thus evident that its mineralocorticoid activity is inadequate to replace that of aldosterone. That 18-hydroxycorticosterone binds poorly (0.2% compared to aldosterone) to renal mineralocorticoid receptors in man [139] and the rat [140] is in agreement with lack of salt retaining action.

A second effect of the steroid has been shown recently on the total output of titratable hydrogen ions by the urine of adrenalectomized rats. Doses as low as 3 μg are effective in increasing this variable. Urinary pH remains constant after the injection of such a low dose, but decreases in animals treated with 6 μg [138]. The possibility that corticosteroids may be directly involved in the control of acid balance has yet to be examined.

RESEARCH TRENDS AND PERSPECTIVES

It is a privilege of reviewers, usually denied to "vulgar authors of ordinary papers", to speculate freely on how current knowledge in their field may be linked together and on possible future developments in their field. Several as yet unexplored aspects of the structure and function of 18-hydroxycorticosterone have already been mentioned. The nature and structure of the "true" precursor of aldosterone, the biological action of 18-hydroxycorticosterone and how it is accomplished at molecular level, and the isolation of receptors from the kidney, a probably target organ, are obvious examples for basic research. The role of 18-hydroxycorticosterone in the control of acid base balance in health and disease will be of considerable interest in medicine. However, perhaps the most fascinating opportunities for interdisciplinary research lie in the nature of the chemical structure of the compound itself, the bonds in the C18-oxygenated pregnane steroids and the relationship of these to the highly specific physiological effects of the steroids, effects which are crucial to the survival of higher vertebrates.

The C18 oxidised pregnane derivatives are unique among corticosteroids in that they exhibit oxo-enol tautomerism under physiological conditions. The weak, covalent O—H bond of the enol (hemiketal) structure facilitates reversible condensation reactions and some of the products of these reactions have chemical and probably also biological properties which differ from their parent steroid compounds. Other weak bonds such as hydrogen bonds, although not yet unequivocally demonstrated, probably exist and other reversible interconversions—tautomeric or otherwise—occur spontaneously depending on environmental conditions such as the structure, composition and proton concentration of the solvent. Moreoever, electronic delocalisation in these 18 oxygenated steroids is not limited to the 3-oxo-4 ene conjugated double bond system of the A ring, but occurs also in the oxygens of the C18 $\rightarrow$ 20 hemiketal and the C18 $\rightarrow$ 11 hemiacetal. Such physicochemical properties may have an important role to play in the organisation and control of metabolism in higher vertebrates and the reader is referred to comprehensive reviews on this subject [141–143].

In mammals, as in most other vertebrates, the adrenal cortex and its homologues are the only steroidogenic glands essential for survival. Of the two main functions which the corticosteroids perform it is the mineralocorticoid activity which contributes to the composition of the "milieu intérieur" and preservation of osmotic and possibly pH homeostasis. It may not be a coincidence that the peculiar physical chemistry of these compounds makes them "molecular structures particularly suitable for being the vehicle of life" [141].

ADDENDUM

Aragonés *et al.*[37] have recently reported a study of the L forms of 18-hydroxycorticosterone. They found that, in acidic aqueous and acidic alcoholic solutions, at least five less polar fractions originated from the more polar parent compound (M form). They also report that components with identical chromatographic mobilities may have different structures. Thus material formed in neutral methanol, which is either a C20 methoxy derivative or a mixture comprising mainly this derivative (see 'Chemistry' section) migrates with an R_M (migration relative to M form) of 4.33. A second compound with an identical R_M originates in aqueous HCl and is a dehydration product or a mixture of dehydration products of the M form. Other fractions have not yet been identified. Interestingly, the second compound (or mixture) mentioned above could be converted spontaneously into aldosterone in a Krebs–Ringer medium containing NADP in the absence of adrenal tissue [144].

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