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# VERSATILE STEROID MOLECULES AT THE END OF THE ALDOSTERONE PATHWAY

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**Summary**—18-hydroxycorticosterone converts spontaneously and reversibly to a variety of less polar forms and derivatives, some of which are precursors to aldosterone. In particular, 21-hydroxy-11$\beta$,18-oxido-4-pregnene-3,20-dione (18-DAL) is hydroxylated to aldosterone with high yields in the presence of malate and $NADP^+$, at pH 4.8. 18-DAL also behaves as a metabolic intermediate between 18-OH-B and aldosterone according to time-course and trapping experiments. Consequently, the final steps of the aldosterone pathway at pH 4.8 could be identified as 18-OH-B,18-DAL and aldosterone, in this sequence. The submitochondrial distribution of aldosterone biosynthesis is compatible with this postulate. The work also shows that some forms of 18-OH-B are promoters of hydrogen transport in renal tubuli and that this regulation may be independent of sodium reabsorption. These results suggest a regulatory model, new in steroid biology, according to which steroid molecules bearing an oxidized angular C18-methyl may undergo structural changes between precursor ("P") and hormonal ("H") forms in response to homeostatic requirements.

## AN INTERDISCIPLINARY APPROACH TO A CONTROVERSIAL FIELD

A number of apparently independent studies have emerged during the past decades from or around the transient instability of steroids that bear an oxidized angular methyl in the C-18 position. Especially 18-hydroxy-pregnanesteroids, but also aldosterone, as reported recently, undergo spontaneous, mostly reversible conversions into less polar forms and derivatives when submitted to acid media and/or relatively polar organic solvents. These conversions have for many years troubled analytically minded steroid chemists but not much attention has been paid for a quarter of a century to their structural nature or mechanism, let alone to their biosynthetic or physiological implications. In the last two decades it has become evident that these transitions play an important role in the biosynthesis of aldosterone and that their modulation may be involved in the regulation of the *milieu intérieur* not excluding acid-base homeostasis. Experimental evidence in these fields has led to an integrative regulatory scheme of unsuspected extension and heterogeneity, the very interdisciplinary nature of which makes it necessary to review certain basic knowledge together with recent findings.

This work intends to introduce investigators of many heterogeneous disciplines related to aldosterone and the *milieu intérieur* to "the other side of the coin" and then to review recent progress in aldosterone biosynthesis and in acid-base metabolism. It finally submits an integrative regulatory hypothesis, new in steroid biology, according to which proton-mediated structure changes or "transitions" may be of homeostatic relevance.

## THE LAST STEPS OF ALDOSTERONE BIOSYNTHESIS

A considerable number of pathways can be predicted from early precursors to the highly oxidized hormonal end product aldosterone, most of which have been demonstrated experimentally. Reference in this respect is made to the authorized reviews and papers by Neher[1], Müller[2] and the highly critical work of Vinson *et al.*[3]. Curiously, these schemes omit $\Delta_5$ pathways. However, Ceballos *et al.* (Ceballos, Gros, Harnik and Lantos, unpublished) have recently found that an "all $\Delta_5$ pathway" is not only present but most probably predominant in the South American toad *Bufo arenarum* H. But in mammals, early[4] and recent[5] evidence favor corticosterone as a mediate precursor although none of the other pathways can be excluded as alternatives in pathological situations. The pathway comprising corticosterone includes 18-OH-B‡ as a theoretical intermediate between B and the mineralocorticoid. However, even if an intermediate role has been demonstrated for 18-OH-B by trapping experiments[4], corticosterone itself showed to be from 17 to 81% more efficient an aldosterone precursor than the usually isolated 18-OH-B form[2, 6]. The extremely poor yields of aldosterone from 18-OH-B were obviously associated to the fact that neither the metabolite nor its substrate are stable

‡18-OH-B and 18-OH-DOC are abbreviations for, respectively, 18-hydroxy-corticosterone and 18-hydroxy-11-deoxycorticosterone. ADX, adrenalectomized rats. 18-DAL or 18-deoxyaldosterone and synonyms of 21-hydroxy-11$\beta$,18-oxido-4-pregnene-3,20-dione.

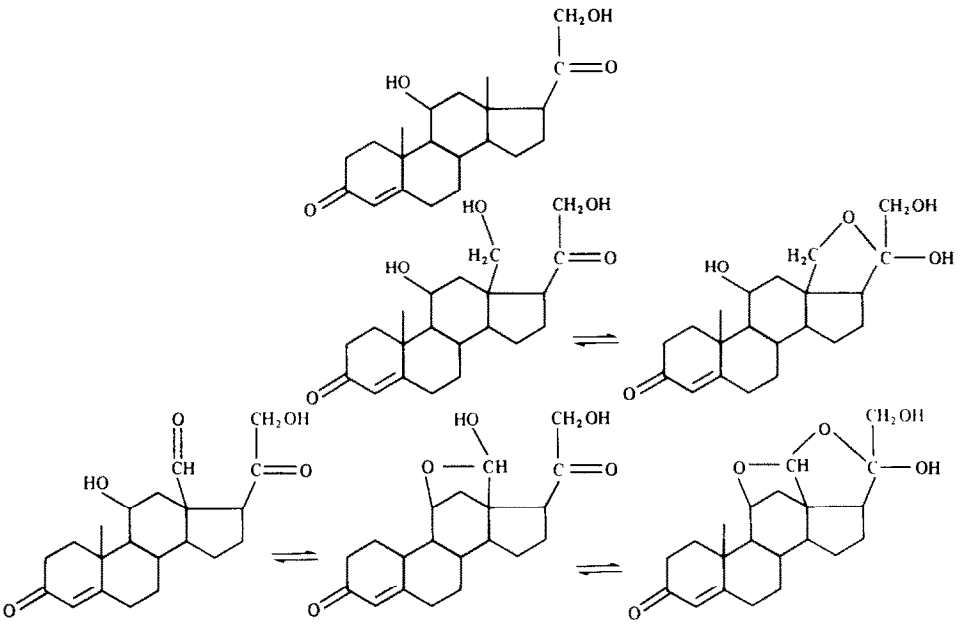


Fig. 1. Corticosterone and isomeric forms of 18-OH-B and aldosterone.

under their open forms. The following scheme explains this hypothesis. (Fig. 1).

## 18-OH-B, ALDOSTERONE AND THEIR "LESS POLAR" FORMS. (a) STRUCTURAL-BIOSYNTHETIC ASPECTS

### *The problem*

When isolated from columns, paper or TLC, 18-OH-B is stable in its $C_{18}$–O–$C_{20}$ hemiketalic forms the so called "M" forms [7]. On the other hand, aldosterone was long believed to be present only under its $C_{11}$–O–$C_{18}$ hemiacetalic forms with traces of the open one. But according to recent studies, employing proton [8] and $^{13}C$ (Burton, unpublished) NMR, aldosterone is an almost equimolar mixture of this form and the bicyclic one represented in Fig. 1.

This problem of monocyclic vs bicyclic forms of aldosterone is of course of foremost biosynthetic importance: the $C_{18}$–O–$C_{20}$ cycle of 18-OH-B "M" is opposite to the $C_{11}$–O–$C_{18}$ ether previously thought to be the predominant structure of aldosterone and this difference was the main reason invoked for the poor precursorship of the former. With the recent findings mentioned above this reasoning lost part of its argument but can still not be discarded to explain, at least a low reaction rate from 18-OH-B to aldosterone. Another possible reason for the poor precursorship of 18-OH-B M is its high polarity which makes difficult its transport through (subcellular or cellular?) membranes to the site of the enzyme action.

### *Structures and precursorship*

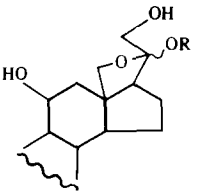
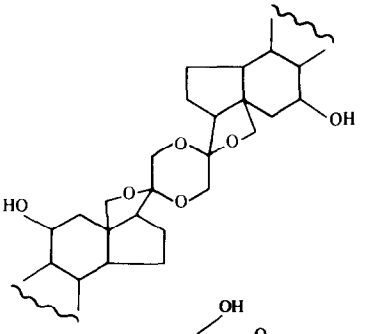
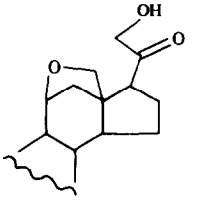
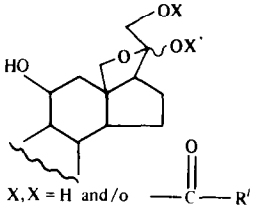
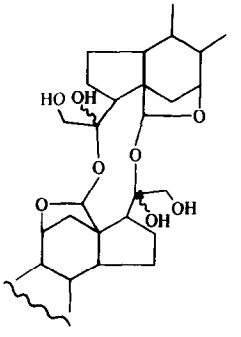
At its beginning [9] ours was an entirely empirical approach to the problem. When analyzing the radioactive metabolites of pregnenolone in the Antartic seal we found: (a) no aldosterone under the *in vitro* conditions employed; (b) considerable yields of 18-OH-B; (c) the ability of 18–OH-B to convert spontaneously and reversibly to a less polar fraction upon storage in methanol, a property observed by others [10] and analyzed in detail in 1965 by Dominguez in the 18-hydroxylated analogue 18-OH-DOC [7]. (d) On the other hand, like others, we found a poor precursorship of 18-OH-B for aldosterone.

It occurred to us at this stage that this less polar, "L" fraction mentioned in (c) could be a better precursor than the usually isolated 18-OH-B M. By then we had already obtained a 10% conversion of L to aldosterone, against less than 4% for M aldosterone [11].

At this point it became clear that we had to review the still scarce bibliography on L structures and to undertake updated experiments on all these L forms and derivatives of 18-OH-B, regarding their number, preparations, optimal stability conditions, structures and of course precursor properties. At present, old and new experimental evidence leads to the following panorama: (see also Table 1).

The low electron density at the hemiketal oxygen of the $C_{20}$ labilises its hydrogen which is easily replaceable by alkyl radicals upon reaction with

Table 1. Less polar forms and derivatives of 18-OH-B

| FORMATION | NAME AND MOBILITY | STRUCTURE | REVERSION | BIOL. PROP |
|---|---|---|---|---|
| Spont. Methan (days) | $R_M$ 4.33 |  | Water (hours) | |
| Microb. from B<br>Spont. Methanol ?<br>$H^+$ | 18-OH-B Dimer |  | | |
| Aq. HCl $N^{-2}$ (inst.) | 18-DAL $R_M$*4.33 |  | Aq.$OH^-$ (inst.) | ALDO → PRECURSOR, Na |
| idem | $R_M$ 1.84 | | Aq.$OH^-$ | |
| idem | $R_M$ 5.26 | | Aq $OH^-$ | |
| idem | $R_M$ 6.34 | | Aq.$OH^-$ | |
| idem | $R_M$ 7.12 | | | |
| Metabolic | SM Solv. front |  | $H^+$ (hours) | Reservoir ? |
| Metabolic (Spont Aldo) $H^+$ | Aldo-Dimer Solv. front |  | | TRANSPORT ? |

$\overline{Na}$: Aldosterone antagonist. Spont.: spontaneous. Microb.: microbiological. Solv.: solvent. Aq.: aqueous. Inst.: instantaneous. $R_M$ mobility relative to M in the Bush $B_5$ system. For details, see the text.

alcohols of different chain length. Under as yet ill defined conditions this hydrogen, and that of the contiguous hydroxyl at $C_{21}$, may even be replaced by a second 18-OH-B molecule [12]; (see also [11]). These molecules constitute one family of L derivatives.

The other family of spontaneously formed hydrophobic compounds originates in a proton-mediated dehydration and inversion of the hemiketalic cycle from the $C_{18}$–O–$C_{20}$ to the $C_{11}$–O–$C_{18}$ position. The decrease in polarity of these compounds with respect to M is mainly due to the functionalization of the $C_{11}$

hydroxyl upon which 18-DAL, the best known member of this family, is obtained. These compounds originate in aqueous acid media: at pH 2 the dehydration is practically instantaneous but occurs also, although to a lesser extent, at pH 4.8.

Table 1 also includes two hydrophobic compounds formed metabolically when 18-OH-B is incubated with rat adrenals. The formation of an ester is not exclusive of 18-hydroxylated steroids. The dimer of aldosterone is formed spontaneously from the mineralocorticoid in acid media and appears as an enzymic reaction product in incubation with 18-OH-B.

Early studies on the $C_{11}$–O–$C_{18}$ ether formed in acid media were made by Kondo [12] and Kirk [13]. This structure was also assigned tentatively to compound $R_M^*$ 4.36 (Mobility relative to 18-OH-B M in the Bush $B_5$ system) obtained upon suspension of the mother steroid in 0.01 N HCl as one of two alternatives ensuing from the interpretation of mass spectra [14]. $R_M^*$ 4.36 was definitely identified as 18-DAL several years later by Proton and $^{13}C$ NMR spectroscopy in the hands of Burton and Cozza[15].

As to the precursorship of the ether for aldosterone, this property was predicted by Neher[1], Ulick[16], Finkelstein[17] and Müller[18]. In fact, Ulick obtained experimentally a 3.8% conversion of exogenously added 18-DAL to aldosterone by frog interrenal tissue [16].

In other experiments, 18-DAL and M were found to be converted *spontaneously* to aldosterone in the presence of NADP [19].

After all these predictions, structure elucidations and isolated precursor experiments the situation became ripe for a systematic although still exploratory investigation in search of aldosterone precursorship among the following factors: Nature of the precursor (18-OH-B M or 18-DAL); reaction (spontaneous or total (spontaneous + enzymatic)); enclosure ("nude" or enclosed in adrenal lyposomes); pH (7.4, 4.8 or 3.3); incubation time (1 h or 2 h).

The rationale behind these factors and their combinations can be found in preceeding sections but some conditions need further explanations: Thus slightly heteropolar organic solvents utilized in previous work for the obtention of the first family of L (see above and [11–14]), were here replaced by total lipids from adrenal cells because it was thought that these lipids constituted a better model for the cellular medium, perhaps even for the situation in cellular or subcellular corticoadrenal membranes.

The results of combining these factors on aldo-precursorship can be seen in Table 2: when a 5 factor ANOVA is applied to these results, 7.4 is found to be a less efficient pH for conversions than more acid hydrogen potentials. Interactions between factors evidence two equally optimal combinations: "18-DAL-total-pH 4.8" and "M-Spontaneous-pH 3.3". We might come back later to the bizarre second

Table 2. Precursor conversions to aldosterone

| Enclosure | Type of conversion | Precursor | Time (hours) | pH 3.3 | pH 4.8 | pH 7.4 |
|---|---|---|---|---|---|---|
| Nude | Total | M | 1 | 6.93 ± 2.21 | 6.64 ± 0.76 | 0.67 ± 0.02 |
| | | | 2 | 9.45 ± 2.37 | 7.12 ± 0.33 | 0.73 ± 0.01 |
| | | 18-DAL | 1 | 4.92 ± 1.43 | 8.66 ± 0.71 | 4.26 ± 0.02 |
| | | | 2 | 5.88 ± 1.52 | 9.66 ± 0.53 | 5.57 ± 0.06 |
| | Spontaneous | M | 1 | 3.60 ± 0.50 | 1.03 ± 0.15 | 1.00 ± 0.02 |
| | | | 2 | 5.04 ± 1.71 | 1.32 ± 0.05 | 1.18 ± 0.03 |
| | | 18-DAL | 1 | 3.65 ± 0.58 | 2.65 ± 0.13 | 4.37 ± 0.02 |
| | | | 2 | 5.21 ± 1.07 | 3.13 ± 0.12 | 5.70 ± 0.04 |
| Liposomes | Total | M | 1 | 1.72 ± 0.06 | 3.88 ± 0.27 | 2.66 ± 0.19 |
| | | | 2 | 1.89 ± 0.22 | 4.42 ± 0.36 | 3.81 ± 0.11 |
| | | 18-DAL | 1 | 1.68 ± 0.23 | 5.68 ± 0.45 | 2.01 ± 0.04 |
| | | | 2 | 2.88 ± 0.27 | 7.02 ± 0.97 | 2.89 ± 0.13 |
| | Spontaneous | M | 1 | 9.90 ± 0.67 | 7.32 ± 1.10 | 1.62 ± 0.02 |
| | | | 2 | 10.45 ± 1.43 | 7.42 ± 0.92 | 1.68 ± 0.11 |
| | | 18-DAL | 1 | 6.78 ± 0.68 | 5.41 ± 0.50 | 3.61 ± 0.37 |
| | | | 2 | 8.12 ± 1.02 | 6.33 ± 0.60 | 5.47 ± 0.08 |

Values are means of triplicates ± Standard Errors. A 5 factor ANOVA discloses differences ($P < 0.01$) within factor pH, disappearing upon elimination of pH level 7.4. Factor time is significant for the remaining pH levels. Fourth order interaction enclosure-conversion-precursor-pH is significant for time = 2 h. ——— Equally optimal results. - - - - - - - - - Results too dispersed to be significantly different. (See also [15].)

interaction and concentrate now on the first one:

If one calculates the purely enzymic component of this conversion (by deducting spontaneous from total yields) and if this component is then compared to its similar one at pH 7.4 one finds a pronounced optimum at 4.8. At the neutral proton concentration the enzymic reaction from 18-DAL to aldo is negligible, conversions at these concentrations being entirely due to the spontaneous component. A thorough analysis of Table 2 furnishes much more information but such an analysis exceeds by far the scope and length of this paper.

The second part of this work was aimed at demonstrating that 18-DAL was not only efficient as exogenous precursor but that it was an intermediate on the pathway B–18-OH-B–Aldo in rat adrenals, at a proton concentration of pH 4.8. This was demonstrated by classical trapping experiments shown on Table 3 and by the time-course of 18-DAL and aldo formations, and disappearance rate of 18-OH-B when the precursor was incubated with these adrenals (Fig. 2).

The conclusion at this point of the study was that *18-OH-B is converted rapidly and spontaneously to 18-DAL in acid medium, that this conversion reverts at a neutral or basic pH and that proton concentrations at or around pH 4.8 are optimal for enzymic transformations of 18-DAL to aldosterone. Furthermore, that at this pH the spontaneous conversion of 18-OH-B to 18-DAL and the enzymic one, from 18-DAL to aldosterone are the last two sequential stages on the aldosterone pathway.*

Since the enzymic conversion of 18-DAL to aldosterone implies a hydroxylation step involving NADPH, molecular $O_2$ and the cytochrome $P_{450}$ chain, we carried out experiments in which homogenates of rat adrenals were incubated in Tris buffer at pH 4.8, supplemented with NADP and increasing concentrations of malic acid–malate. At a 9 mM concentration of malate, a 16% yield of aldosterone from 18-DAL, and a slightly lower one from 18-OH-B, were obtained in 90 min (Table 4). These represent the highest yields so far attained employing any form or derivative of 18-OH-B surpassing by

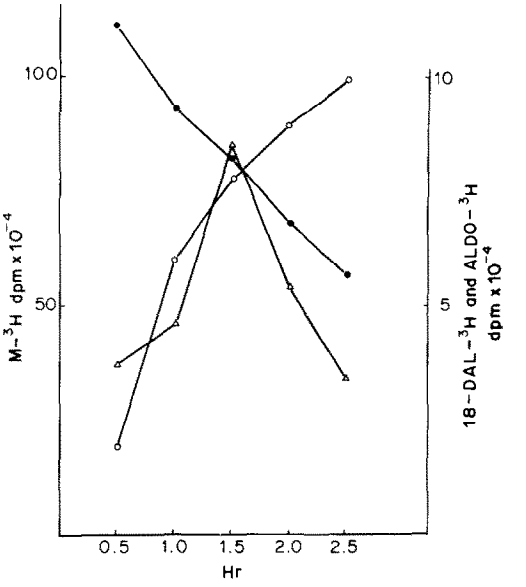



Fig. 2. Disappearance rate of 18-OH-B M and time-course of 18-DAL and aldosterone. ●——● 18-OH-B; △——△ 18-DAL; ○——○ Aldo (Adapted from [15]).

50% even the usual conversions seen for *corticosterone* to aldosterone. All these findings allow us to write the following reaction sequence (Fig. 3.).

This sequence evidences a dual necessity for pro-

Table 3. Trapping experiments at pH 4.8

| Trapping metabolite | | % Aldo from B-$^3$H | % Aldo from M-$^3$H |
|---|---|---|---|
| M (ng) | 0 | 11.3 | |
| | 1 | 8.0 | |
| | $10^2$ | 4.9 | |
| | $10^4$ | 2.6 | |
| | $10^6$ | 1.7 | |
| 18-DAL (ng) | 0 | 10.5 | 7.0 |
| | 1 | 7.1 | 6.2 |
| | $10^2$ | 3.7 | 4.3 |
| | $10^4$ | 1.3 | 2.1 |
| | $10^6$ | 0.5 | 0.5 |

B: corticosterone. See [15] and the text for details.

Table 4. Effect of cofactors and malate on the production of aldosterone from M and 18-DAL at pH 4.8

| Malate (mM) | NADP 0.5 mM | NADPH 0.5 mM | % Aldosterone-$^3$H from M-$^3$H | % Aldosterone-$^3$H from 18-DAL-$^3$H |
|---|---|---|---|---|
| — | — | + | 4.98 | 5.84 |
| — | + | — | 2.98 | 4.62 |
| 0.15 | + | — | 5.37 | 6.56 |
| 0.60 | + | — | 6.45 | 8.92 |
| 1.20 | + | — | 8.67 | 11.24 |
| 3.00 | + | — | 11.58 | 15.67 |
| 9.00 | + | — | 13.27 | 16.22 |
| 9.00 | — | — | 7.48 | 8.09 |
| — | — | — | 3.24 | 4.41 |

See the text and [15].

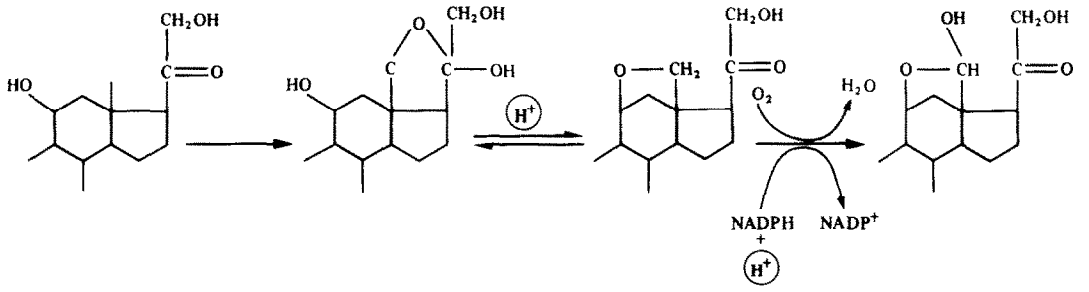



Fig. 3. Reaction sequence from corticosterone to aldosterone.

tons thereby furnishing an explanation for the conversion optimum at pH 4.8. It is not clear however whether the enzyme activity is optimal *only* because the precursor is stabilized at pH 4.8 or whether the hydroxylase possesses also *per se* a maximal activity at this pH.

A hydroxylation step from 18-OH-B to aldosterone has been demonstrated by the group of Marusic[20] and later by Aupetit *et al.*[21], Kojima *et al.*[22] and Ulick[23]. Wade recently demonstrated the identity between both 18-hydroxylases[24]. Our experiments are in line with these ideas and findings but maintain that *at least in one of several pathways at low pH levels, 18-OH-B is first transformed into 18-DAL and that 18-DAL is the immediate hydroxylation substrate.*

### *Is 4.8 a "physiological" pH? Submitochondrial enzyme sites*

Obvious questions arising from the above conclusions were whether those relatively high proton concentrations exist within the cell and whether aldosterone precursors reach acid cell zones. It is now well established that acidity levels within certain organelles such as lysosomes[25] and receptosomes[26] attain a pH between 4 and 5. Commonly a hydrolytic role is ascribed to these pH levels. However, aldosterone is biosynthesized not in those particles but in mitochondria[27]. Since the interior of mitochondria is itself highly organized, no single pH can be assigned to the whole. But after having fallen into disgrace for many (too many) years, Mitchell's chemiosmotic hypothesis postulating an electrochemical gradient and translocation of protons across the inner membrane[28] is now universally accepted. One hypothesis suggests high proton densities in a narrow layer close to the outer face of this inner membrane.

We fractionated mitochondria by the method of Schnaitman *et al.*[29] in which a 0.5% digitonin solution is utilized to separate Mitoplasts from the remaining organelle. The outer portion was in turn fractionated into Outer Membrane and Intermembrane Space by centrifuging in Hepes at 100,000 **g** for 60 min[29a]. The following enzyme markers were used to assess the purity of submitochondrial fractions: Succinic dehydrogenase, for Mitoplasts; Monoamine Oxidase, for External Membrane; and Adenylate Kinase, for Intermembrane Space. These sub-mitochondrial fractions were incubated in the presence of [$^3$H]corticosterone under conditions of enzyme saturation. Table 5 shows that the total activity of enzyme markers could be recovered from the expected fractions.

Table 5. Submitochondrial distribution of aldosterone biosynthesis and enzyme markers

| | | | | Aldosterone | |
|---|---|---|---|---|---|
| Fraction | SDH | AK | MAO | Malate + NADP$^+$ | NADPH |
| M | 31.775 | 3.32 | 101.5 | 17.3 | 15.2 |
| T | 27.138 | 0.00 | 7.2 | 8.4 | 6.3 |
| E | 841 | 3.14 | 90.3 | 1.3 | 4.4 |
| X | 726 | 0.04 | 86.7 | 0.4 | 0.8 |
| I | 56 | 2.99 | 0.7 | 0.4 | 0.4 |
| T + E | | | | 9.6 | |
| T + X + I | | | | 7.6 | |

M: Mitochondria. T: Mitoplasts. E: Outer Mitochondria comprising Interspace and External Membrane. X: External Membrane. I: Interspace. SDH: Succinic dehydrogenase. AK: Adenylate kinase. MAO: Monoamine oxidase. Activity of enzyme markers is expressed in enzyme units. Aldosterone is expressed in nmoles formed from 260 nmol. [$^3$H]Corticosterone in 20 min. For details see the text.

A detailed analysis of 18-hydroxylase activities in this experiment is of interest because it gives us an idea on the aldosterone synthesizing efficiency of individual subfractions as well as binary and tertiary mixtures of these subfractions. The results were the following (Table 5): (a) Significant hydroxylase activities could be found in Mitoplasts and in the Outer Mitochondria. (b) Neither fraction, nor their mixture, matched the activity of the organized mitochondrion. (c) Interspace and the Outer Membrane, incubated separately, lack the activity exhibited by the whole outer mitochondrion.

These findings respond fairly to a hypothesis conferring to Interspace (to its proton layer close to the outer face of the inner membrane) the ability to form 18-DAL from precursors, and to Mitoplasts, as well as Outer Membrane, hydroxylase activities [29a].

## 18-OH-B, ALDOSTERONE AND THEIR LESS POLAR FORMS; (b) BIOLOGICAL ASPECTS

Under a variety of conditions, 18-OH-B has been found in relatively abundant quantities in pulmonary vertebrates of aquatic habitat such as ducks [30], water turtles [31] and seals [9]. In these species aldo/18-OH-B ratios are low. Even if there is no evidence for its particular abundant production by amphibian vertebrates, Ulick's early studies on 18-OH-B were performed on frogs [32]. These early observations elicited investigations relating 18-OH-B to acid-base homeostasis.

### *The problem*

The renal elimination of protons is a most vital process in pulmonary vertebrates of aquatic habitat. Species phylogenetically far apart such as ducks and penguins in *aves*, dolphins, pinnipeds and whales among mammals and water turtles among reptiles compensate low oxygen pressures during apnea through converging strategies such as specific hemoglobin structures, sensitivity to 2,3-diphosphoglycerate and others. But how do those higher vertebrates resist acidosis resulting from high partial $CO_2$ pressures? By adapting to acidosis and by enhancing the hydrogen-eliminating capacity of their kidneys. It is well known that H-elimination depends to a great extent on Na–H exchange.

In higher vertebrates this interchange occurs at two levels: In proximal tubules, where H secretion depends on a passive sodium transport through mucosae, i.e. an apical Na gradient; and at distal levels, where proton elimination depends on a luminal negative potential originating in Na reabsorption. But Na–H interchange turns from a blessing into harm when sodium retention and extracellular expansion are undesirable.

From a biological viewpoint, there are therefore no reasons to believe that H elimination should *universally and primarily* depend on sodium retention. Fortunately other processes of proton secretion coexist with Na–H interchange. Important among these processes are $CO_2$ reabsorption, mainly at the proximal level, and an electrogenic ATP dependent, Na independent active transport of $H^+$ ions by medullary collecting ducts in mammals. Water turtles and toads possess these "proton pumps" abundantly in the urinary bladders, which are believed to be homologous to those distal structures of mammals. A third family of Na-independent, H eliminating processes consists of "the trapping" of H ions. Protons are trapped in the lumen either by filtered buffers or by ammonia synthesized by the tubular cell in an adaptive response to an acid load.

### *Corticoadrenal involvement in acid–base homeostasis*

The multiplicity of H eliminating mechanism is matched by the multiplicity of actions of each family of corticoids. Moreover, in this field conclusions seem to depend more than in other fields on the experimental model: Results obtained in the whole animal are not always reproducible in studies with isolated nephron segments or membrane vesicles from tubular cells, nor are the integrated findings necessarily coherent. Also, even close related species respond differently to identical steroids and chronical treatments differ often from acute ones. Last but not least, individual adrenal steroids seem to be unable to restore totally acid–base parameters altered by adrenalectomy.

Taking the *adrenalectomized Kagawa rat* as a model to which corticosteroids were administered *acutely* we undertook in the late seventies a systematic investigation on corticoidal effects upon parameters related to acid–base homeostasis. We utilized in our studies physiological doses of corticosterone, aldosterone and 18–OH-B [33].

In this model corticosterone increased the elimination of phosphates, ammonium and slightly that of potassium; and aldosterone decreased, as expected, the elimination of sodium. Most, if not all these results were in good agreement with those of others working on different models with chronical injections of steroids [34–37].

However, in our model only 18-OH-B affected urinary acid–base parameters. We administered the steroid at three dose levels and found a dose-dependent increase in urinary titratable acidity. At the highest dose employed 18-OH-B also decreased urinary pH. The sodium-response was bimodal: 18-OH-B *increased* urinary sodium elimination at a dose level of 3 $\mu$g and decreased this elimination at 6 $\mu$g [33]. These results should be compared to those of Lanthier and Sandor, who showed that 18-OH-B enhanced sodium elimination by the nasal gland of ducks [38], and an early report, according to which the steroid almost lacks sodium-retaining properties in the rat [10].

We also used adrenalectomized rats to investigate the effects of aldo and 18-OH-B on blood pH, $pCO_2$ and bicarbonate levels. The experiments were car-

ried out under normal respiratory conditions and under atmospheres containing 20% $CO_2$. In these experiments only 18-OH-B alcalinized blood (in normally breathing animals) or avoided an excessive acidification (under high-$CO_2$ atmospheres). The latter experiment is shown in Fig. 4 [39].

Thus, *in our experimental model*, the administration of 18-OH-B offered protons, and that of corticosterone, proton acceptors to the nephral lumen. In other models a role in hydrogen elimination was also assigned to aldosterone and, to a lesser extent, to glucocorticoids. But, according to the present evidence, only 18-OH-B enhances hydrogen elimination independently of sodium retention.

It is practically impossible to review, in this paper, the numerous investigations on the site of corticosteroid action and the often conflicting conclusions on the mechanisms through which these steroids promote H elimination. (See [40] and [41] for reviews). There is a general agreement to assign to the distal nephron a role of "fine adjustment" in electrolyte transport. (Type I) receptors for aldosterone are located at the distal convoluted tubule (DCT), the cortical-(CCT) and the medullar collecting tube (MCT); Na, K, Mg dependent ATPases are located at the basal membrane of CCT, where aldosterone exerts its control on sodium retention by increasing ATP levels at the site of these ATPases and by promoting the apical diffusion of Na towards the cell. But *proton dependent ATPases* are located at MCT and there aldosterone exerts its second action, specifically on H elimination. (See [40] for a review).

Damasco and Malnic have recently ventured into the proximal tubule, a nephral segment generally unfavored by experimental endocrinologists due to its lack of aldosterone receptors, to study the effects of the steroid trilogy on luminal acidification. They experimented on control and sham operated specimen, on ADX and on ADX to which either corticosterone, aldosterone or 18-OH-B were administered. In their experiments they used a stopped-flow microperfusion technique and pH microelectrodes to measure tubular acidification kinetics. Parameters measured, or calculated, were stationary pH, acidification half time ($t_{1/2}$), initial and final bicarbonate levels and net $HCO_3^-$ absorption ($J_{HCO_3}$). The authors found that adrenalectomy caused an increase in proximal luminal pH from 6.78 to 7.02 and an increase in $t_{1/2}$ from 4.4 s to 11.4 s in consequence $J_{HCO_3}$ fell dramatically from 2.2 to 0.5. The administration of any of the three steroids to ADX rats decreased $t_{1/2}$ and increased $J_{HCO_3}$ without reaching control values. Their effects on stationary pH, however, were more selective: only aldosterone and 18-OH-B, but not corticosterone, were able to decrease luminal pH values, again without reaching control levels [41].

These results are of interest from various points of view. For one thing, they offer certain similarities with the above-mentioned experiments in the whole rat. One remarkable coincidence between both experimental series is the lack of a decrease in urinary (or luminal) pH after corticosterone treatment. This characteristic differentiation of the glucocorticoid from the two other hormones may well be due to an increased $NH_3$ synthesis by the kidneys of corticosterone-treated rats.

The authors also postulate an alternative (or additional?) mechanism according to an analog model appropriated for their stopped-flow method. In this model [42], an increase of $t_{1/2}$ represents a decrease in the number of apical transport sites, which in the proximal tubule are mostly Na/H exchangers. This hypothesis is strengthened by the results of Kinsella *et al.* who found that glucocorticoids were necessary for the stimulation of proximal Na/H exchange by metabolic acidosis [47].

As a result of these experiments and those of others (see for example [48]), it can be stated that adrenal steroids accomplish a complementary role in the rat's acid–base homeostasis.

*Glucocorticoids* increase GFR and probably Na/H exchange at the proximal apical membrane. *But above all, they offer proton acceptors to the lumen, all along the nephron.*

*Aldosterone* affects Na/H exchange by two mechanisms in the distal nephron, and also Na/H interchange at the proximal level. It also stimulates Na independent H transport in the medullar collecting duct; (this transport has been demonstrated in the rabbit).

*18-OH-B* stimulates H elimination by an Na-independent mechanism. The nature of this mechanism is still unknown, but, given the steroid's site of action at the proximal tubule, proximal ATP dependent proton transport independent of Na/H antiport, recently reviewed by Kinne–Saffran and Kinne [43], is in this sense of particular interest.

### *Conclusions and trends towards a new integrated model in steroid biology*

Taken separately, the results of the above sections emphasize the role of less polar forms and derivatives of 18-hydroxylated $C_{21}$ steroids, as well as the role of an acid pH (cell zones with high proton densities) in the biosynthesis of aldosterone from 18-OH-B. They also demonstrate the complementary actions of adrenal steroids in the maintenance of acid–base homeostasis and stress the importance of 18-OH-B in the Na-independent stimulation of H elimination.

From an integrated, albeit still speculative viewpoint these results may be assembled into a regulatory model that exhibits certain similarities with the allosteric regulatory model of Jacob and Monod. In the proposed model, *precursor molecules*, not enzymes, would experience reversible structural changes or transitions between forms with hormonal activity (H forms) and forms with biosynthetic (precursor) properties (P forms). Equilibria between H and P forms would be displaced in response to

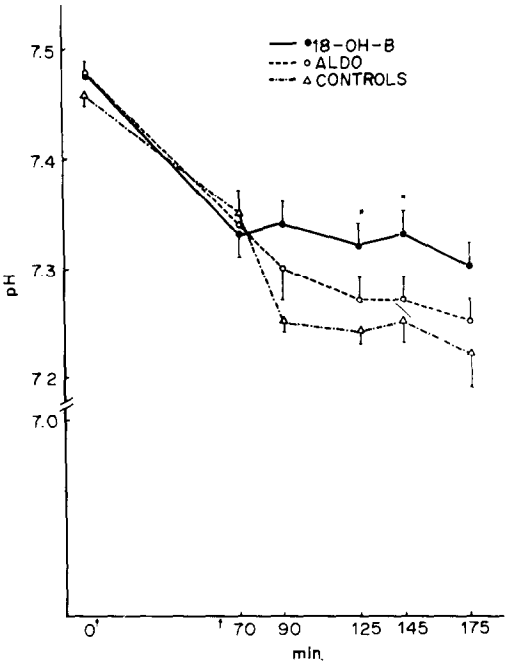



Fig. 4. Blood pH of rats under atmosphers containing 20% $CO_2$. △——△ adrenalectomized controls. ○——○ aldosterone-treated. ●——● 18-OH-B treated. 1st arrow: 1st injection of steroids and introduction into high $CO_2$ atmosphere. 2nd arrow: 2nd injection of steroids. *Different from controls $P < 0.025$. Means of 8 rats ± standard errors (adapted from [39]).

external stimuli, noxious or others, and in accordance with homeostatic requirements. 18-DAL (and perhaps other L forms according to Table 1) would represent the P forms, while 18-OH-B M would be the H form possessing an effect on Na independent proton elimination; but L forms other than 18-DAL (Table 1), specially those formed from M in very acid media, should not be excluded from this hormonal function. Lichtwald's ideas on H eliminating effects of the aldosterone dimer are in general agreement with this scheme [44].

In the H-P model, the step 18-DAL—aldosterone is of additional interest. Indeed, 18-DAL is not only a precursor to, but also an antagonist of aldosterone [45]. The stimulation of this enzymic step (See Fig. 3) would imply an accumulation of a sodium retaining steroid and the disappearance of its antagonist, while the inhibition of the same step would have the opposite effects.

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