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SOME COMPARATIVE STUDIES IN ADRENOCORTICAL STEROIDOGENESIS: AN INTERPRETATION OF THE FUNCTIONAL HOMOLOGIES OF THE MAMMALIAN AND NON-MAMMALIAN ADRENAL CORTEX

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SUMMARY

Using a technique of incubation with dialysis of products, steroid synthesis by adrenal tissue from the skink, *Tiliqua rugosa*, and the fresh water teleost *Coregonus clupeoides* was compared with the rat adrenal *z. glomerulosa*. [^{14}C]-Acetate and [^{3}H]-pregnenolone were used as precursors throughout. The preparations showed a number of similar features.

1. In each case, aldosterone and corticosterone were prominent products formed from both precursors.
2. Two pools of steroid products were demonstrated, a free and a bound pool. Typically, [^{14}C]-labelled steroids were bound and [^{3}H]-products were more freely dialysable. In the rat, *z. glomerulosa* and the *Coregonus* incubations, the bound steroid was released by the addition of an appropriate ACTH preparation.
3. The two pools of steroid appeared to have different metabolic fates. The free pool gave rise predominantly to corticosterone in the rat and *Tiliqua* and generally had a high $^{3}H/^{14}C$ ratio. The bound pool gave rise to aldosterone which had a low $^{3}H/^{14}C$ ratio. In the rat *z. glomerulosa* and in *Coregonus*, the release of bound steroid caused by ACTH was associated with a decrease in yields of aldosterone relative to glucocorticoid formed from [^{14}C]-acetate.

Neither the formation of aldosterone nor the ACTH-sensitive compartmental arrangement of steroid is found in the rat inner adrenocortical zones. In common with other features these findings suggest comparability of non-mammalian adrenocortical function with the mammalian *z. glomerulosa*.

INTRODUCTION

The morphology of the adrenal cortex has been studied extensively using light and electron microscope techniques. In general the differentiation of at least three adrenocortical zones, the *zonae glomerulosa, fasciculata* and *reticularis* is found throughout the metatherian and eutherian mammals, whereas in the non-mammalian vertebrates (while there are small areas of ambiguity) the overall impression is that adrenocortical tissue is made up of uniform, homogeneous cells undifferentiated into zones[1–3].

The structural and functional homologies are not entirely clear. On structural grounds it is difficult to draw conclusions regarding the affinities of the homogeneous adrenocortical tissue of a non-mammalian vertebrate with any particular zone(s) of the mammalian gland, in part because even among the mammals there is wide variation in zonation[2, 3]. It is possible that ultrastructural criteria may throw further light on this point, since the mitochondria of the zona glomerulosa of the mammal are different in form and internal structure from those of the inner zones[4–7]. However, this may also be subject to considerable species variation[7].

On functional grounds the differences between the mammalian *z. glomerulosa* and the *fasciculata/reticularis* are more clear cut, viz:

1. Only the *z. glomerulosa* is a source of aldosterone and, in many species, the 17-deoxysteroids[8–17].
2. Compared with the response of the inner zones, *z. glomerulosa* stimulation by ACTH is relatively slight [11, 16–27].
3. In contrast to the inner zones, which respond only to ACTH[17, 30–32] the *z. glomerulosa* may be stimulated by additional circulating factors including K^+, serotonin and angiotensin II, and possibly other substances which respond to changes in electrolyte balance [16, 17, 21–34, 74].
4. In contrast to the inner zones, steroids in the *z. glomerulosa* of the rat adrenal cortex appear to be

maintained in at least two pools during biosynthesis, a free and a bound pool. These have different metabolic fates and the free steroid pool gives rise largely to corticosterone, whereas the bound is more susceptible to 18 hydroxylation, leading to the formation of 18-hydroxycorticosterone and aldosterone. The bound pool is only seen in products formed from endogenous precursors, and not from a late pathway added precursor such as [^{3}H]-pregnenolone. Interchange between the pools may be governed by ACTH[35–38]. Such pools may be domonstrated simply by incubating adrenal tissue in dialysis bags. The method has two special characteristics in that it not only enables a distinction to be made between the pools on the grounds of dialysibility, but it also results in enhanced conversion of the bound steroid as compared with the free, and thereby exaggerates any differences in their metabolic fates.

In order to determine the functional homologies of the adrenal cortex in mammalian and non-mammalian vertebrates, it seemed worthwhile comparing patterns of steroid production and dialysibility in incubations of non-mammalian adrenals and rat adrenal *zona glomerulosa* tissue, to examine whether the compartmental arrangement of steroids characteristic of the *z. glomerulosa* is also seen in non-mammals. This paper presents results obtained in two such species, the teleost *Coregonus clupeoides* and the reptile *Tiliqua rugosa* and compares findings with rat *z. glomerulosa* results.

MATERIALS AND METHODS

Animals

Rats were obtained from commercial suppliers and maintained for brief periods in the Department of Physiology, Queen Elizabeth College. *Coregonus clupeoides* was collected during a stay at the Field Station of the Zoology Department, University of Glasgow, situated on Loch Lomond. Fish were caught be seining, stored briefly in keep nets, but killed for incubation of adrenal tissue within 1–2 h. *Tiliqua rugosa* was collected in its natural habitat in South Australia and kept for periods of up to a few days in the Department of Animal Physiology, Waite Agricultural Research Institute, University of Adelaide.

Incubations

Adrenal tissue was incubated in bags of dialysis tubing as previously described[35]. In the case of the rat incubations, *z. glomerulosa* tissue was separated by crushing the glands and discarding tissue other than the capsule which remains intact. Incubations were performed with tissue from control and from cortisol treated rats, and in some instances ACTH (2.V. Synacthen, Ciba) was added to the incubations of the tissue from cortisol treated animals. With *Coregonus* incubations of minced head-kidney tissue from untreated animals were carried out, in some cases in the presence of Synacthen or of *Coregonus* pituitary extract. With *Tiliqua*, incubations were performed with minced whole tissue from control and dexamethasone treated animals, and Synacthen or *Tiliqua* pituitary extracts were added to some of the latter.

In all cases, [1-^{14}C]-acetate (0·75 μCi/ml: S.A. 61 mCi/mmol) and [7α-^{3}H]-pregnenolone (0·06 μCi/ml: S.A. 6·9 Ci/mmol) were used as added precursors. Isolation and quantitation of the products followed established procedures of extraction, chromatography in several systems, the formation of derivatives and conventional isotope dilution techniques[138, 101–103].

RESULTS

Yields of steroids

Coregonus. The major steroids produced from both precursors were cortisone, 11-deoxycortisol, corticosterone and aldosterone (Fig. 1). 17-hydroxysteroids were prominent, but cortisol was present in too small quantities to allow definite characterization, presumably indicating the presence of an active 11β-dehydrogenase converting cortisol to cortisone. Comparisons of the incorporation of the labels suggest little difference in the steroid profiles formed from the two substrates.

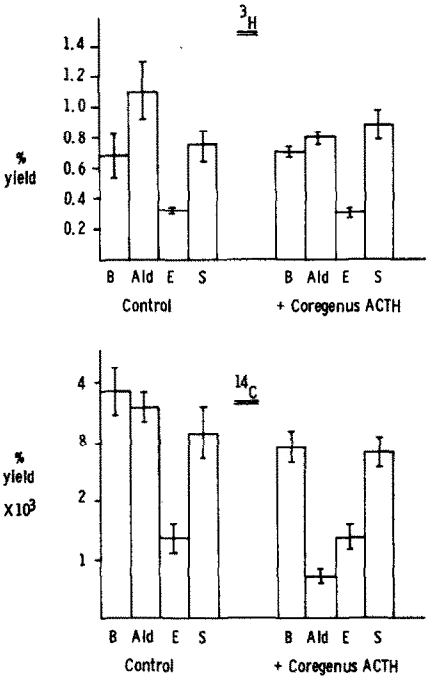

Fig. 1. *Coregonus* adrenocortical products from [^{14}C]-acetate and [^{3}H]-pregnenolone in the presence and absence of *Coregonus* pituitary extract. B = corticosterone, Ald = aldosterone, E = cortisone, and S = 11-deoxycortisol. Comparison of [^{14}C]-aldosterone in control and ACTH groups, $P < 0{\cdot}001$.

Tiliqua. Two steroids were examined, aldosterone and corticosterone. Deoxycorticosterone was not formed to any appreciable extent. In contrast to the *Coregonus* incubations, there appears to be a difference in the metabolic fates of the two precursors, in that yields of aldosterone and corticosterone were of the same order from acetate, but considerably more corticosterone than aldosterone was formed from pregnenolone. This is reflected in the order of magnitude difference in the $^{3}H/^{14}C$ ratios for corticosterone and aldosterone (Fig. 2).

Rat z. glomerulosa. Four major products were studied, deoxycorticosterone, corticosterone, 18-hydroxydeoxycorticosterone and aldosterone. Deoxycorticosterone and corticosterone showed relatively high $^{3}H/^{14}C$ ratios, whereas those for 18-hydroxydeoxycorticosterone and aldosterone were much lower, again suggesting that the two precursors were handled differently (Fig. 3).

Dialysibility of steroids

Coregonus. Compared with the theoretical distribution, in which a freely dialysable steroid would show only 12% inside and 88% outside the dialysis bag under the

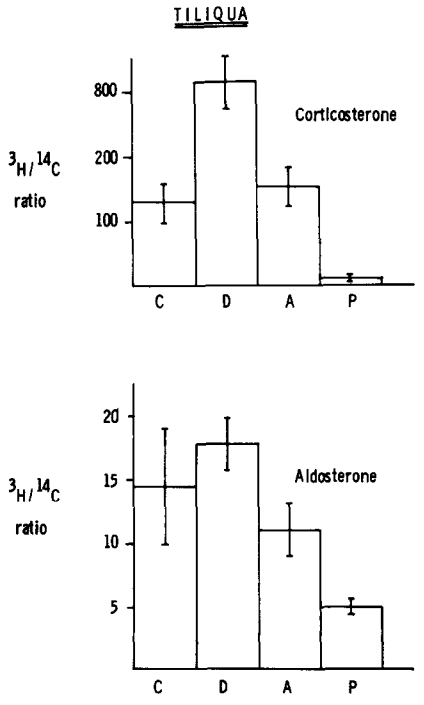

Fig. 2. *Tiliqua* adrenal product $^{3}H/^{14}C$ ratios after incubation with [^{14}C]-acetate and [^{3}H]-pregnenolone. C = control, D = dexamethasone pretreated, A = dexamethasone pretreated with the addition of Synacthen to the medium, P = dexamethasome pretreated with the addition of *Tiliqua* pituitary extract to the medium. *P* values: corticosterone, C vs D < 0·01; C vs P < 0·01.

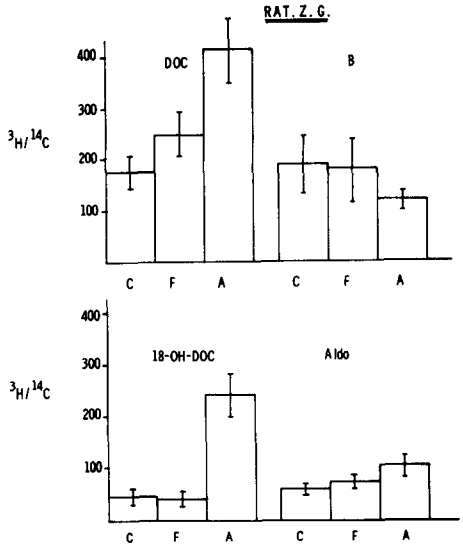

Fig. 3. Rat *z. glomerulosa* product $^{3}H/^{14}C$ ratios following incubation as for other species (Figs. 1, 2). DOC = deoxycorticosterone, B = corticosterone, 18-OH-DOC = 18-hydroxydeoxycorticosterone, Aldo = aldosterone. C = control, F = cortisol pretreated, A = cortisol pretreated with the addition of Synacthen to the incubation medium. *P* values: DOC, C vs A < 0·01; 18-OH-DOC, C vs A < 0·01; Aldo, C vs A < 0·05.

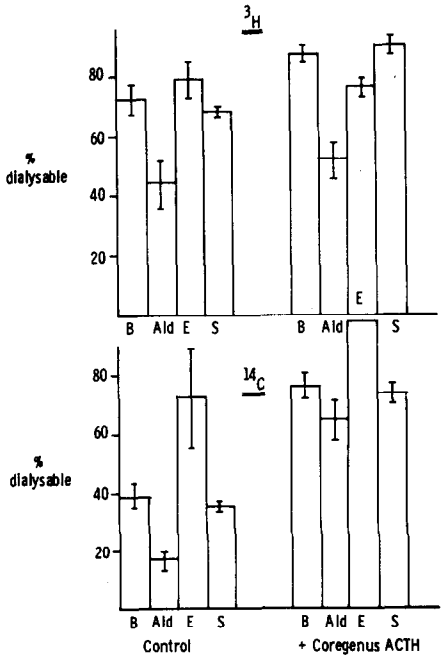

Fig. 4. *Coregonus* adrenal product dialysibility under conditions as for Fig. 1, abbreviations as Fig. 1. *P* values, [^{14}C]-B, control vs ACTH < 0·001; [^{14}C]-ald, control vs ACTH < 0·001; [^{14}C]-S, control vs ACTH < 0·001.

conditions used in these experiments, all steroids showed some degree of binding with less than 88% penetrating the dialysis membrane. With the single exception of cortisone, ^{14}C-labelled products were less dialysable than the ^{3}H steroids (Fig. 4).

Tiliqua. In this species there was a clear distinction between the ways in which the [^{14}C]- and [^{3}H]-labelled pools were handled by the tissue. Throughout all the experimental procedures both [^{3}H]- and [^{14}C]-corticosterone was virtually freely dialysible at all times. In contrast, whereas [^{3}H]-aldosterone was freely dialysible, [^{14}C]-aldosterone characteristically showed slightly, but significantly less dialysibility at most times (Figs. 5a and b).

Rat z. glomerulosa. In this species too there appeared to be a clear distinction between the way the [^{14}C]- and the [^{3}H]-labelled steroid was handled. All [^{3}H]-labelled steroids were freely dialysible at all times. (Figs. 6a and b). In contrast, the [^{14}C]-labelled steroids were significantly less dialysible than their [^{3}H]-equivalents at least in the absence of ACTH. This effect was particularly marked for 18-hydroxydeoxycorticosterone.

Effects of ACTH

Coregonus. Coregonus pituitary extract, but not Synacthen, effected at least a partial release of corticosterone 11-deoxycortisol and aldosterone from the

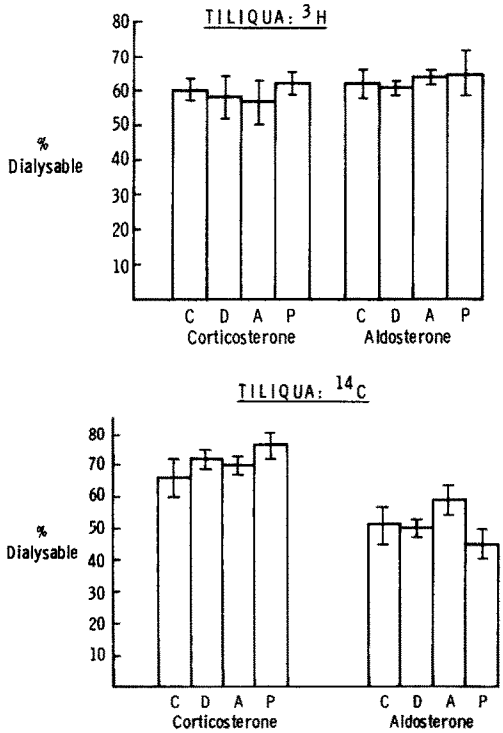

Fig. 5. *Tiliqua* adrenal product dialysibility under conditions as for Fig. 2, abbreviations as Fig. 2. *P* value for total [^{14}C]-aldosterone vs [^{3}H]-aldosterone < 0·05.

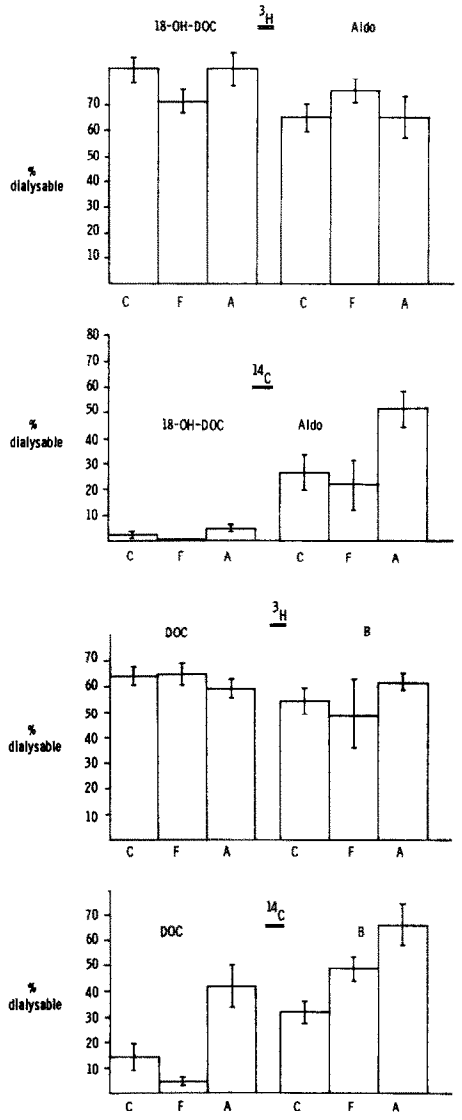

Fig. 6. Rat *z. glomerulosa* adrenal product dialysibility under conditions as for Fig. 3, abbreviations as Fig. 3. *P* values: [^{14}C]-DOC, C vs A < 0·02; [^{14}C]-aldo, C vs A < 0·05: [^{14}C]-18-OH-DOC, C vs A ≐ 0·05.

bound condition. This was more pronounced for the [^{14}C]-labelled steroids than for the [^{3}H]-steroids in each case (Fig. 4). It was associated with a dramatic decline in yield of [^{14}C]-aldosterone: other products were relatively unaffected.

Tiliqua. Neither dexamethasone pretreatment, nor the addition of *Tiliqua* pituitary extract or Synacthen affected the dialysibility of any of the products (Figs. 5a and b). Relative yields were affected as shown by the changes in ^{3}H/^{14}C ratios (Fig. 2), with a marked increase with dexamethasone pretreatment and a

decrease with pituitary extract. These effects are more marked in corticosterone than aldosterone.

Rat z. glomerulosa. The addition of Synacthen to the incubation medium greatly increased the dialysibility of all the bound [^{14}C]-labelled steroid (Figs. 6a and b). The dialysibility of all [^{3}H]-labelled steroids was unaffected. At the same time the ^{3}H/^{14}C ratio of all the products except corticosterone was increased (Fig. 3).

DISCUSSION

Compartmental arrangement of steroids

The results presented here show some remarkable points of similarity between adrenocortical function in the two non-mammalian species *Coregonus* and *Tiliqua*, and the *z. glomerulosa* of the rat adrenal.

In the first place, all three preparations produced aldosterone from both precursors (Figs. 1–3). Secondly, some points of difference emerge between the profiles of steroid formed from [^{14}C]-acetate and from [^{3}H]-pregnenolone. In *Tiliqua* and rat *z. glomerulosa* incubations, similar yields of aldosterone and corticosterone were formed from [^{14}C]-acetate, but under control conditions substantially less aldosterone relative to corticosterone was formed from [^{3}H]-pregnenolone. This is reflected in the relatively low ^{3}H/^{14}C ratios for aldosterone (Figs. 2 and 3). This suggests a different biosynthetic origin for these products, and one possibility is that they are formed from separate pools of precursor under control conditions. If it is assumed that the acetate more readily penetrates and reflects conversion of the endogenous precursor pool, it may be that in general relatively more aldosterone is formed from endogenous precursors than from a late pathway exogenous precursor such as pregnenolone. This certainly appears to be the case in the rat *z. glomerulosa* and in *Tiliqua* adrenocortical tissue[35–38, 104]. (Steroid formation from endogenous precursors in *Coregonus* has not yet been assessed because of the special problems this tissue presents). It may even be that the conversion of [^{3}H]-pregnenolone is spurious, and the yields of [^{3}H]-products have no significance.

The concept of two pools for [^{3}H]- and [^{14}C]-labelled steroids is borne out by their different properties of dialysibility. In all cases, the major "glucocorticoid" (cortisone in *Coregonus*, and corticosterone in the other two species) was relatively freely dialysible at most times, for both labels. In contrast, other products were bound to some degree and this was always more marked for the [^{14}C]-labelled steroid than for the [^{3}H]-steroid. The hypothesis may therefore be developed that the bound pool of steroid, as exemplified by the steroid products from [^{14}C]-acetate, are more readily transformed into aldosterone than into glucocorticoid, whereas the free steroid is more readily converted into the glucocorticoid. Changes in dialysibility effected by ACTH may therefore be expected to result in changed proportions of products in the steroid profile: this was true both in the case of the rat *z. glomerulosa* (Fig. 3) and also in *Coregonus* (Fig. 1). It is probable that purely quantitative data may be uncertain in the dialysis incubations since any release from binding effectively dilutes the steroid by a factor of ten, as it becomes free to penetrate the outer pool of the incubation flask. It is unlikely that stimulation of absolute yields can always be recognized under these circumstances but changes in relative proportions of products are clearly apparent. In the rat *z. glomerulosa* incubations, the results give further information about the possible pathway to aldosterone. In tissue from control and cortisol pretreated animals, the ^{3}H/^{14}C ratios for 18-OH-DOC and aldosterone are much lower than for DOC and corticosterone (Fig. 3). This suggests the possibility that 18-OH DOC and aldosterone are biosynthetically related, at least in being formed from the same pool of precursor and it may even be that the bound 18-OH DOC is a direct precusor of aldosterone. Only in the ACTH group in which the [^{14}C]-DOC and [^{14}C]-18-OH DOC are released from binding (Fig. 6) does the ^{3}H/^{14}C ratio of 18-OH DOC rise, presumably because less bound [^{14}C] DOC is then available for 18-hydroxylation. This change in ^{3}H/^{14}C ratio is also seen to a lesser extent in aldosterone. The selective effect of ACTH on corticosterone is reflected in its fall in ^{3}H/^{14}C ratio which contrasts with the rises in the ^{3}H/^{14}C ratio of the other products.

The relative lack of effect of Synacthen in the *Coregonus* and *Tiliqua* preparations is not unexpected since the ACTH native to these species is known to differ from mammalian ACTH even in the 1–24 region[105]. The lack of effect of *Tiliqua* ACTH on the adrenal binding of [^{14}C]-aldosterone in this species appears to be inconsistent with the results for the other two species, however data on steroid production from endogenous precursors shows in this case a difference between ^{14}C and unlabelled steroid: the unlabelled steroid shows more characteristic changes in dialysibility becoming less dialysible in the tissue from animals with pituitary ACTH secretion suppressed by corticosteroid treatment, and being released with the addition of *Tiliqua* ACTH to the incubation medium (104). Thus, in this species all three precursors behave somewhat differently from each other and the situation is complex. Nevertheless, central points of comparison with the other preparations used in this study remain clear. In addition, a selective stimulation of corticosterone by ACTH in the *Tiliqua* incubations may, as in the rat incubations, be deduced from the large changes in ^{3}H/^{14}C ratio: increased following dexamethasone pretreatment and decreased with the addition of Synacthen or *Tiliqua*

pituitary extract. Aldosterone shows something of the same effect, but it is much less marked (Fig. 2). Thus, although this specific effect on corticosterone is not correlated with any apparent change in the compartmental arrangement of the [^{14}C]-steroid this may simply result from the relative insensitivity of the method in this case.

By no stretch of the imagination are the three species studied at all closely related phylogenetically. It seems to us to be entirely likely that mechanisms similar to those we have described should eventually be found in other species and may be widespread. Certainly in the duck for example, evidence that endogenous and late pathway exogenous precursors may exist in separate pools has already been reported[106]. In addition it is relevant to emphasize that the existence of separate pools and the exchange of steroid between them appears to be connected with the capacity to respond to ACTH stimulation with a specific increase in corticosterone secretion with less or no effect on other compounds such as 18-hydroxydeoxycorticosterone or aldosterone. A selective stimulation of corticosterone secretion relative to aldosterone by ACTH has also been shown in the duck[62, 63] and it is possible that the two phenomena, compartmental arrangement of steroid and selective stimulation of secretion of one steroid rather than another, may be related.

In the amphibia, *Rana catesbiana* and *R. pipiens*, *in vitro* effects of ACTH have been shown to affect aldosterone more than corticosterone, in contrast[107, 108]. However, results obtained *in vivo* in *R. catesbiana* show on the other hand that secretion of corticosterone was more greatly affected than aldosterone by ACTH treatment or by hypophysectomy[48]: there is therefore a capacity for selective stimulation which for some reason does not appear to function in the same way under *in vitro* conditions. This discrepancy is also seen in the relative proportions of the compounds since *in vitro* more aldosterone is produced than corticosterone, whereas *in vivo* the relationship is reversed. A similar discrepancy between *in vitro* and *in vivo* findings occurs in *Tiliqua*, Since the large yields of aldosterone relative to corticosterone seen in these experiments are not reflected in the proportions of circulating steroids *in vivo*[109]. The significance of the large capacity for aldosterone synthesis in the adrenals of these species and possibly of other non-mammalian vertebrates as well (see below) is therefore open to speculation.

Functional affinities of the mammalian and non-mammalian adrenal cortex

The literature suggests that the adrenal cortex of many non-mammalian vertebrate species shows features of mammalian *z. glomerulosa* function. Most striking is the widespread capacity to synthesize aldosterone which has been definitively shown in teleosts[39–42], elasmobranchs[43], amphibia[46–50], reptiles[51–55] and birds[56–66] and indeed in the non-mammalian tetrapods the capacity of adrenal tissue to synthesize aldosterone *in vitro* appears to surpass the mammalian capacity by far[46–67]. Furthermore, the synthesis of aldosterone is always accompanied by the production of other 17-deoxysteroids, including corticosterone, and in the Elasmobranchs and the non-mammalian tetrapods 17-deoxysteroids are produced almost exclusively[46–70] with at best only trace production of cortisol (e.g. Refs. [57–59]).

The response to ACTH appears to be less sensitive than in whole mammalian tissue. In rats and dogs hypophysectomy can result in a 90% decrease in corticosteroid secretion within 2 h[71–74] which may be prevented by infusion with ACTH[71, 72, 75, 76]. The dynamics of the response to ACTH suggest that glucocorticoid output is virtually completely dependent on ACTH stimulation and furthermore that the maximal corticosteroid output attained under ACTH stimulation may be one or more orders of magnitude above the basal level[71, 72, 75, 76]. Among the non-mammalian vertebrates however, acute hypophysectomy often results at best in a 70–80% reduction in circulating corticosteroid and in many cases this may still be true after several days[48, 77–88, 93–95].

The magnitude of the steroidogenic response to ACTH is also significant and it seems clear that the increase in corticosteroid secretion of one or more orders of magnitude have not been recorded in vertebrates other than eutherian mammals. Commonly, increases in the region of 3–4 fold are the maximal response to stress or ACTH treatment[48, 80–84, 89–92]. The area is nevertheless open to debate, and with regard to their studies in the duck, Bradley and Holmes state that "the adrenal cortex in the duck may be even more sensitive to the stimulus of corticotropin than this tissue in mammals." However, even in their experiments, there was a 40 min delay in reaching maximal steroid stimulation by ACTH in acutely hypophysectomized ducks (a much longer period than in the rat for example) and they still obtained responses to ACTH in 14-day hypophysectomized animals [86, 87], whereas a similarly treated mammal would probably show an adrenal completely refractory to ACTH.

The mechanism of control of hormone secretion by the *z. glomerulosa* in mammals is itself far from clear although it is well known that its steroid output relates to changes in electrolyte balance or circulatory changes. Stimulation of adrenal/pituitary activity is often seen in non-mammalian vertebrates in changes of electrolyte

balance although the part played by stimulatory factors other than ACTH is not always apparent[47, 48, 62, 79, 96–100].

In summary it may be concluded that in many respects—the nature of the compounds produced, aldosterone and the 17-deoxycorticoids; the relatively insensitive response to ACTH; the response to changes in electrolyte balance, and the compartmental arrangement of pools of steroid precursors—the adrenal cortex and its homologues in non-mammalian vertebrates show more similarity to the *z. glomerulosa* than to the inner adrenocortical zones of a mammal such as the rat. This concept leads to the speculation that the functions of the inner adrenocortical zones emerged with the evolution of the mammal. It is not difficult to see the special selective advantages this could confer, a high capacity to secrete specifically glucocorticoid hormones combined with a heightened sensitivity to ACTH, confer a powerful capacity to mobilize energy reserves in response to environmental changes and stress. In this context it is pleasing to note that other authors working on the mode of action of corticosteroids, reach the conclusion that the "glucocorticoid" function of the familiar adrenal steroids such as cortisol and corticosterone, as distinct from "mineralocorticoids" such as aldosterone, is only really apparent in mammals and does not occur in the non-mammalian *z. fasciculata/z. reticularis* and the specific response of the target organs to glucocorticoids evolved concurrently.

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DISCUSSION

Ungar:

When you talk about the difference between bound and free pools, does this extend to precursors at the level of cholesterol or even pre-cholesterol which are bound?

Vinson:

It's very difficult to try and see where this binding starts in the biosynthetic pathway. Mostly it's difficult because of the problems involved in incubating the adrenal tissue with cholesterol. I suspect that it may be at the point of cholesterol side chain scission. As pregnenolone emerges from the mitochondria, it may be bound to a protein, but I don't know.

Ungar:

Do you know of any evidence where pregnenolone or steroids beyond that are bound? (Especially in adrenal tissue).

Vinson:

Yes, there was evidence presented last year by Kream and Saver (*Endocrinology* **92** (1973) suppl. Abstract. 113). I think at the Chicago meeting of the American Endocrine Society. Using tritiated pregnenolone they were able to show a specific receptor for tritiated pregnenolone within adrenocortical cells.

Müller:

How can you convert bound aldosterone to free aldosterone? Probably you use solvent extraction but could you also liberate it by displacement with cold aldosterone?

Vinson:

No, because the nature of the binding material, whatever it is, is such that we can't use exogenous steroids from pregnonolone onwards in the pathway and show this kind of binding. I think it does occur but only in very small amounts. So we just use solvent extraction.

Carstensen:

Wouldn't it be interesting to try to investigate the frog, specifically the bull frog. I was able 15 years ago to show that the bull frog adrenal increased its aldosterone production at least 5 times, *in vitro*, with ACTH.

Vinson:

Yes, it does *in vitro* but it doesn't *in vivo* apparently.

Carstensen:

No, apparently not.

Vinson:

We often have a discrepancy between *in vitro* findings and *in vivo* findings in respect to aldosterone production, which may be much higher *in vitro* than one would expect from *in vivo* observations. It is very difficult to assess the significance of this.

Carstensen:

With regard to rat adrenal, have you any idea where in the cells this bound fraction is? Could it be in the lysosomes. I think about 5 years ago Cortes and Peron (*Biochim. biophys. Acta* **126** (1966) 43) showed by some sort of electrophoresis that they could release 99% of corticosterone. They found in electron microscopy that lysosomes were depleted.

Vinson:

Yes, it's certainly a possibility. Once again, the problem in locating this material and characterizing it is that we can't label it in a conventional way with a high specific activity labelled steroid.

Domínguez:

I have noticed that the main effort of steroid biochemistry in recent years has been oriented to the study of the mechanism of action of steroid hormones and everybody is working with target tissues, forgetting a little bit about what is still to be discovered in the general processes of steroid hormone biosynthesis. For example, there is very little information about bound or free precursors and intermediates, about the actual pool size, of various endogenous precursors and intermediates, in organelles of endocrine tissues and less information is available regarding the transport systems across the cell membrane and between organelles in the cells of steroid-producing endocrine tissues. I was wondering, therefore, if someone here has information about the pools of endogenous precursors in steroid biosynthesis in relation with what is being discussed.

Vinson:

We really started working on this topic because of looking at the size of different pools (Vinson and Whitehouse, *Acta endocr. (Kbh)* **16** (1969) (695) and at the same time Lommer *et al.* (*Hoppe-Seylers Z.* **352** (1971) 805 and 1491) were doing the same sort of thing. I think we had compatible results although we reached different interpretations of how it works. The point is this: the pool size of precursors along the pathway to corticosterone, say, the pool sizes of progesterone and deoxycorticosterone are not large enough to account for the massive output of corticosterone if one looks at the way they change with time. You just can not see that movement of steroids through those total precursor pools which one might, perhaps, expect to see.

Sjövall:

If one gives acetate and cholesterol with different labels at a constant rate, can one measure any differences in specific activities with time in these steroids? I mean to place this step of partitioning somewhere.

Vinson:

In the entire animal?

Sjövall:

Yes.

Vinson:

Well, we haven't done it. But we have this problem as always it seems to me of getting cholesterol converted at all.

Sjövall:

Yes, *in vitro*. I was thinking of *in vivo*, isolating metabolites as they come. We have some information on this in that we have given ethanol which has deuterium in the 2-position which is then converted to acetate in the liver and serves as a precursor of cholesterol. From the distribution and content of deuterium in different parts of the steroid skeleton, it is possible to calculate how much of a compound originates from the ethanol. If one does this with cholesterol and corticosterone metabolites in bile one finds that these compounds are formed to the same extent from ethanol and show the same kinetics for deuterium incorporation. This indicates that the cholesterol pools used for biliary excretion and for corticosterone biosynthesis are equilibrated. We do not know anything about the precursor of aldosterone.

Rousseau:

Is the fish you have studied a fresh or sea water fish?

Vinson:

Fresh water.

Rousseau:

Have you found aldosterone in a salt water fish? I ask you this because of the classical view according to which aldosterone has evolved as a result of adaptation to a non-salt-containing environment.

Vinson:

Yes, that is one interpretation. However, in one species, e.g. the eel (Sandor *et al.*, *J. Endocr.* **34** (1966) 105) one finds no aldosterone, whereas in another, e.g. the herring, Truscott & Idler (*Gen. Comp. Endocr.* **13**, (1969) 535) have shown *in vitro* conversion of corticosterone to aldosterone, so it can occur in sea water species. To what extent it is physiologically significant of course one doesn't know. There's no doubt that an animal living in a plentiful supply of sodium may well have a very low aldosterone output. At the meeting in Budapest, Dr. Carlos Lantos *et al.* (*Gen. Comp. Endocr.* (1974) suppl. to vol. 22, abstract 206) showed some of his data obtained with a marine mammal—the Weddell seal—which apparently produces no aldosterone. I think it may well be a question of adaptation, but not of evolution.