The mislabelling of deoxycorticosterone: making sense of corticosteroid structure and function

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Abstract
Over the 70 or so years since their discovery, there has been continuous interest and activity in the field of corticosteroid functions. However, despite major advances in the characterisation of receptors and coregulators, in some ways we still lack clear insight into the mechanism of receptor activation, and, in particular, the relationship between steroid hormone structure and function remains obscure. Thus, why should deoxycorticosterone (DOC) reportedly be a weak mineralocorticoid, while the addition of an 11β-hydroxyl group produces glucocorticoid activity, yet further hydroxylation at C18 leads to the most potent mineralocorticoid, aldosterone? This review aims to show that the field has been confused by the misreading of the earlier literature and that DOC, far from being relatively inactive, in fact has a wide range of activities not shared by the other corticoids. In contrast to the accepted view, the presence of an 11β-hydroxyl group yields, in corticosterone or cortisol, hormones with more limited functions, and also more readily regulated, by 11β-hydroxysteroid dehydrogenase. This interpretation leads to a more systematic understanding of structure–function relationships in the corticosteroids and may assist more rational drug design.

Introduction
Pick up any endocrinology text published in the last 60 years, and if it deals with the actions of the corticosteroids, you will usually read something to the effect that deoxycorticosterone (DOC, 11-deoxycorticosterone, cortexone, 21-hydroxyprogesterone, Fig. 1) is a weak mineralocorticoid that has virtually no glucocorticoid activity (e.g. Baylis 1953, Genest 1955, Nelson 1962, Steele 1975, Hodges 1984, Guyton 1991, Orth & Kovacs 1998, Parker & Rainey 2004, Norris 2007). In fact, neither of these statements is true: DOC is both a potent mineralocorticoid and also a glucocorticoid (Brookes et al. 2011). To the contrary, among the vertebrates and indeed some invertebrates as well, it may have an extraordinary array of functions. Such misleading views may have frustrated rational analysis of steroid hormone structure–function relationships for decades. Quite how this compound has been so misunderstood is an interesting puzzle that takes us back to the pioneer days of steroid hormone discovery.

Beginnings: isolation and the bioassay of corticosteroids
Systematic elucidation of glucocorticoid structure and the study of their actions and physiology started in the 1930s. Corticosterone (Fig. 1) was the first corticosteroid to be isolated from bovine adrenals (Reichstein 1937), and in the same year, Steiger & Reichstein reported the partial synthesis of DOC from Δ5,3-acetoxyetienic acid (Steiger & Reichstein 1937, Shoppee 1964). The following year, Reichstein & Euw (1938) reported its isolation from bovine adrenals. This was at the start of the tremendous burst of activity that showed about 31 steroids isolated by their group from the same source, in the period from 1935 to 1960. Other groups, notably Kendall & Mason at the Mayo Clinic, Wintersteiner & Pfiffner at the Columbia University, and Kuizinga and Cartland of the Upjohn Company, independently isolated some of the same compounds, although these did not include DOC (see Shoppee (1964)).

From the beginning, it was clear that some had misgivings about DOC. Indeed, some thought that its isolation from adrenal tissue might even be an artefact, since by 1950 it had still not been repeated (Selye 1950, Baylis 1953). However, by that time, it had been recognised that little steroid is actually stored within the gland and that, therefore, secreted products were a far better source (Vogt 1943). It was soon shown that DOC is produced by perfused or incubated bovine tissue (Hechter et al. 1951, Plager & Samuel 1954) and that it is secreted in vivo in rats (Vinson & Rankin 1965), dogs (Taylor et al. 1972b) and humans, both as the free steroid and the
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It is worth considering the muscle contraction assays in more detail. Following the observation that repeated contraction of the rat gastrocnemius muscle requires an intact adrenal, Ingle developed this as a bioassay, later adopted by the Kendall group. In this assay, pentobarbital/ether anaesthetised rats were adrenalectomised, and within 1 h of operation, the gastrocnemius muscle was subsequently stimulated to contract while under a load of 100 g. Adrenal extracts and further pentobarbital were injected s.c. in the neck. Survival times and rate of work were recorded at 12 h intervals for a maximum of 120 h (Ingle 1936). Contractions in the weight-loaded gastrocnemius muscle in adrenalectomised rats were also recorded in the Everse & de Fremery assay (Everse & de Fremery 1932). In this case, animals were allowed to recover from the operative stress for ‘a few days’ before experimentation (the regime is not fully described). Groups of adrenalectomised animals were then treated with test materials, notably cortin, again for ‘a few days’. Repeated stimulations over periods of minutes were then recorded in ether anaesthetised animals: here, it was the recovery from fatigue that was measured.

Point is this: although these two bioassays essentially differ only in the period of time over which treatments and measurements are made, they give massively different results. DOC is extremely active in the Everse & de Fremery method; in fact, some 12.5 times as active as corticosterone, but, in the Ingle method, it is virtually inactive, with maximally 4% of the corticosterone value (Ingle 1940a,b, Grundy et al. 1952, Tait et al. 2004). It is likely that the differences are due to the postoperative recovery period allowed in the Everse–de Fremery test and its lack in the Ingle test (Vögötl 1943a). Though such variations between the data obtained in different assays were not unprecedented, the Kendall group

It is important to note that during the period in which isolation and characterisation of steroids was underway, i.e. in the 1930s and 1940s, a wide variety of bioassays were in use. None of the groups that were involved in the isolation of adrenal steroids in the 1930s and 1940s, including those of Kendall and Reichstein, distinguished between ‘glucocorticoids’ and ‘mineralocorticoids’, and these terms were not used in either of the latter two groups’ Nobel Lectures in 1950 (Kendall 1951, Reichstein 1951). Partly this could have been due to the fact that most of the steroids they isolated shared the same activities, which were already known to be of much wider physiological significance than suggested by these narrow terms. Furthermore, the consequences of adrenocortical insufficiency or of Addison’s disease could be completely reversed by administration of just one of them, for example cortisone (Ingle 1950).

Figure 1 The structures of deoxycorticosterone (DOC), deoxycorticosterone acetate (DOCA), corticosterone and aldosterone (in two forms, see Brookes et al. (2011)). According to a prevailing view, corticosterone is primarily a glucocorticoid, aldosterone primarily a mineralocorticoid, and DOC is a weak mineralocorticoid with no glucocorticoid activity. Since corticosterone differs from DOC only by the presence of an 11β-hydroxyl group, but aldosterone has a more complex acetal or hemiacetal structure, these functional designations defy rational interpretation of steroid structure–function relationships.

Since DOC was available through synthesis, its actions were readily studied. Or, rather, its acetate, DOCA, was available (Fig. 1). One of the curiosities of the history of DOC is that it is as the acetate that most of its actions have been investigated. This appears to be because the synthetic route used by Steiger & Reichstein leads first to the acetate rather than to the free compound. Early on, Reichstein (1951), suggested that biologically there was no difference between the two, and for the benefit of a (slightly) simpler and cheaper synthesis, DOCA was made available. To this day, there is sometimes a careless misunderstanding about the distinction between the two compounds, and DOCA is occasionally used wrongly as an abbreviation for DOC itself. Astonishingly, Reichstein (1951) himself was not above (very infrequent) careless use of language, as in his published Nobel Lecture, in the statement ‘deoxycorticosterone (I: referred to here as DOCA)’. The assumption appears to be that in vivo the acetate is rapidly hydrolysed by non-specific esterases to the free alcohol, and hence it is the latter that binds to the receptor. This may well be the case, although it has not been widely verified (Grekin et al. 1980). The likely sites of deacetylation and the identities of the enzymes involved would reward study.

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had no particular reason to believe that DOC is an important hormone, as noted above it had not been isolated in their laboratory and there was doubt about whether it was actually a secretory product.

This appearance of inactivity was compounded when the data were compared with gluconeogenic results.

**Gluocorticoid activity**

It was Britton and Silvette who first showed that the adrenocortical extracts of Hartman and Swingle and Pfiffin could restore the low blood glucose concentrations of adrenalectomised rats (Britton & Silvette 1931) and that they also increased blood sugar and liver and muscle glycogen in intact animals, particularly in young fasted rats and rabbits (Britton & Silvette 1932). Later, the source of the glycogen was shown to be protein catabolism (Long et al. 1940, Venning et al. 1946). These activities define the use of the term ‘glucocorticoid’ introduced by Selye & Jensen (1946) to distinguish this class of steroids from the ‘mineralocorticoids’, which were characterised by their stimulation of sodium and chloride retention (see below).

In an early study on the effects of the newly available crystalline steroids, blood glucose was assayed in partially pancreatectomised rats and in completely pancreatectomised/adrenalectomised animals (Long et al. 1940). All were treated for 3–5 and 5–10 days postoperatively. DOC (not the acetate) was tested only on the partially pancreatectomised group and cortisone only in the pancreatectomised/adrenalectomised group, whereas corticosterone treatments were used in both groups. It is actually not clear what effect ‘partial’ pancreatectomy (about 30% of the pancreas was removed) has on basal blood glucose levels, but, unsurprisingly, it was very obvious that corticosterone had a much more pronounced effect in animals that had been completely pancreatectomised as well as adrenalectomised. Despite the fact that DOC was not tested on adrenalectomised animals, the authors concluded that DOC had only a feeble effect compared to the other steroids (Long et al. 1940).

A ‘definitive’ bioassay based on gluconeogenesis was developed by Reinecke & Kendall (1942). In this assay, male rats (140–160 g) were adrenalectomised and given 1% saline orally with standard laboratory food for 3 days, at which point food was withdrawn. After 1 day of fast, samples to be assayed were injected s.c. at hourly intervals, and finally, animals were anaesthetised 1–2 h after the last injection, and livers were removed and frozen prior to glycogen assay. It was for convenience that treatment periods were limited to 7 h, although it was known that longer periods gave more glycogen deposition. This became the standard method adopted in America and elsewhere, with usually very little if any modification (Olson et al. 1944a,b, Venning et al. 1946, Pabst et al. 1947). This method showed that activity of adrenal extracts could be replicated by cortisol, cortisone, corticosterone or 11-dehydrocorticosterone administration, but DOCA was inactive (Reinecke & Kendall 1943).

Later, direct comparisons were made between the muscle work assay of Ingle and the glycogen deposition assay using adrenalectomised rats (Pabst et al. 1947). In this study, although corticosterone, 11-dehydrocorticosterone, cortisone and cortisol were tested extensively, DOCA received relatively scant attention. At a dose of 2 mg in total (twice the highest dose of the other steroids), the value for liver glycogen (expressed as a percentage of liver wet weight) was 0–02%, compared with 1–2% + for the other steroids. Clearly, this is indeed a very weak effect. It is nevertheless somewhat odd in that it appears to have been decided almost at the start that DOCA was not really worth pursuing: a single dose was used for this steroid alone, and no statistics are given. Comparison between the two assay methods was made for the other steroids, but not for DOCA.

At this time, there was thus a pervasive view, at least in the US, that DOC was hardly worth wasting time over, and in many cases, tests on its activity were quite perfunctory. Nevertheless, this was seen as sufficient. In his Nobel Lecture, Kendall (whose group had not isolated DOC) stated that it ‘did not appreciably affect the activity of muscle or the metabolism of carbohydrate and protein’ (Kendall 1951) but of the references he quoted only Long’s paper (Long et al. 1940) supported this view. Even in the early 1940s, Kendall’s statement could have been seen to be wrong in both aspects, not only in view of DOC’s potency in the Everse–de Fremery muscle contraction test noted above but also because its effectiveness in elevating blood urea led Olson et al. (1944a) to use it as their standard for comparison with adrenal extracts. It also very effectively stimulates weight gain in intact and adrenalectomised rats, whereas adrenal extracts or cortisone reduce it (Olson et al. 1944a, Conway & Hingerty 1953): neither paper records food intake. Therefore, its activity, apparently combining increased protein turnover with an overall anabolic effect, is entirely unique, not shared by any other steroid, and it has to be said, not properly explored to this day.

In retrospect, therefore, this general lack of interest in DOC is really quite surprising. DOCA, as one of the first of the corticosteroid derivatives to be synthesised, was early shown to be able to maintain adrenalectomised dogs (Thorn et al. 1938), and it was also one of the first to be used in patients with adrenocortical insufficiency or Addison’s disease (Ferrebee et al. 1939, Thorn et al. 1939a,b, Soffer et al. 1940). It was remarkably effective, indeed Thorn et al. (1939a,b) concluded that it was effective as an adrenocortical extract. However, because it had no effect on glucose tolerance curves, Soffer et al. (1940) concluded that it had no effect on carbohydrate metabolism. What this actually showed, of course, is an absence of effect on sensitivity, or resistance, to insulin. Indeed, others later took the point further and showed that DOCA actually enhanced insulin sensitivity (Cheng & Sayers 1949), see also Burns et al. (1992). All this suggests that a systematic study of the actions of mineralocorticoids and glucocorticoids...
on glucose tolerance would be rewarding, but it does not appear to have been done.

Perhaps it should not therefore come as a surprise to learn that during the same period others demonstrated a clear gluconeogenic effect of DOC. The main protagonist was Professor Fritz Verzár whose distinguished career covered a wide field, including nutrition and ageing as well as endocrinology, over a scientifically active span of about 72 years (Zs.-Nagy 2006). His adrenal studies started in collaboration with Reichstein (Verzár 1939) and were later conducted within his own group. Much of the thrust of Verzár’s findings centres on the point that the magnitude of the gluconeogenic effect of DOCA in liver depends critically on the timing and duration of the treatment. However, the studies are more complex. First, Montigel & Verzár showed that glycogen phosphorylation, which was reduced in skeletal muscle from adrenalectomised cats (or dogs), was restored by DOC pretreatment, though DOCA was weaker. Importantly, they attributed this disparity to the weak capacity of skeletal muscle to hydrolyse DOCA. Corticosterone (either as the free steroid or as the acetate) and cortisol (as the acetate) were less active (Montigel & Verzár 1943a). Again using adrenalectomised cats, they then showed complete restoration of normal liver and muscle glycogen by DOCA (the authors describe it as desoxycorticosterone, but indicate that the source was Percorten (Ciba), which was in fact the acetate, DOCA). Typically, animals were maintained on DOCA treatment for a week after adrenalectomy, and treatment was then withdrawn for a further 5 days, after which the test doses of DOCA were administered for a further 3 weeks (Montigel & Verzár 1943b). Similar effects were found in adrenalectomised rats in which it was confirmed that in treatment over the short time span of just a few hours, DOCA had no glucocorticoid effect, in contrast to cortin or cortisol. Over a treatment period of 2–28 days on the other hand, the gluconeogenic effect of DOCA was highly significant, and it restored liver glycogen values to the levels in normal protein-fed animals and in animals on normal or high-glucose diets, though requiring 50% higher doses in animals on a pure protein diet (Vöglini 1943b, Sass–Kortsak et al. 1949). It was nevertheless possibly less potent than cortisol overall (Wang & Verzár 1949). In these studies, muscle glycogen was somewhat less affected. Verzár & Wenner (1948) had already shown that glycogen was reduced by DOC in isolated rat diaphragm muscle, indicating glycogenolysis and, in this way, DOC opposed the actions of insulin. In later in vivo studies, this was found to be dose dependent, and in adrenalectomised rats (sustained for 3 days on 3 mg DOCA per day, then untreated for 24 h), a single dose of up to 2 mg DOCA was glycogenic, whereas 10 mg was less effective, and 20 mg inhibitory (Verzár & Wang 1950). DOCA thus stimulates both gluconeogenesis and glycogenolysis, highlighting again the complexity of its actions.

Other authors supported the general thesis. Nissim (1952) showed a definite, if weak, gluconeogenic response to DOCA in vivo, even in the short experimental time period of 6 h. Good gluconeogenic responses to DOC were also obtained in rat and rabbit liver slices in vivo (Chiu 1950). Somewhat later, it was suggested that in vivo inhibition of muscle glycogen formation was attributable to the potassium loss (Niedermeier & Carmichael 1960, 1962), which DOC certainly also causes, though see later discussion. Looking at other metabolic intermediates, DOCA, like cortisone acetate, restored levels of hexose phosphates, phosphocreatinine and ATP in skeletal muscle from adrenalectomised rats (Conway & Hingerty 1953).

None of this would have deterred Ingle. Using his 1945 article in Annual Reviews in Physiology as a suitable vehicle for polemic, he first launched a fierce defence of his muscle contraction assay. This had come under attack by Vöglini (1943b), who pointed out that the operative methods were extremely stressful, that muscle work (and liver glycogen) was depressed even in sham operated animals, and that provided sufficient treatment time of >2 days was allowed, DOCA was as effective as cortin (Vöglini 1943a, b). Among other things, Ingle (1945) claimed that Vöglini had simply ignored his (Ingle’s) data on adrenalectomised rats treated for 7 days (Ingle 1940a). It is interesting to compare these data sets. Using 1 mg DOCA/day for 2–10 days, Vöglini obtained work data identical to the unoperated controls. Using 2 mg DOC or DOCA per day for 7 days, as far as one can judge (data from unoperated animals and adrenalectomised DOCA-treated animals are depicted in different figures), Ingle in fact also obtained recovery to unoperated control values. It is important to note that, at this time, data presentation was by no means consistent, and the now universal mean±S.D. format was not always used, nor was the use of even simple statistical tests such as Student’s t-test (although the methods were available). Instead, Ingle showed bar charts with mean values indicated, but with all the data points individually included. Vöglini simply tabled all the values. So the matter of whether data were significantly different or not was up to the judgement of the author (or reader). Replotting their data (as far as one is able) in the modern convention confirms that their results were actually identical (Fig. 2). It is difficult not to conclude that either Ingle misinterpreted his own data, or that he could not accept a legitimate challenge to firmly held views. He even concluded: ‘It is the opinion of the reviewer that the data of these (Vöglini’s) papers do not seriously challenge the generally accepted conclusion that 11-desoxycorticosterone differs qualitatively in its biological properties from the C–11 oxygenated compounds’, i.e. does not restore the capacity of muscle to do work in the adrenalectomised animal. There is no doubt, however, that in the standard Ingle muscle work method, the animals were highly stressed. He wrote: ‘Ingle has repeatedly emphasised that prolonged stimulation by a severe shock is required to test the endurance of the animal’ – this in animals that were first tested an hour after adrenalectomy! These animals were not merely stressed, they were literally destroyed – the procedure was continued in this way for up
to 120 h or until the animal succumbed. (One may doubt whether current ethical concerns would allow such procedures today). Turning then to the stimulation of gluconeogenesis by DOC observed by Montigel and Verzar, he stated dismissively that ‘These conclusions are opposed by the results of many careful investigations from other laboratories and clinics’ and ‘...it has been established beyond reasonable doubt that the compound 11-desoxy-corticosterone is deficient in its ability to stimulate the formation of new carbohydrate from non-carbohydrate sources’ (Ingle 1945).

It was with these *ex cathedra* pronouncements that further interest in DOC’s actions was effectively killed.

Among other things, this had the odd effect that other workers were thus led to believe that the Ingle test assayed glucocorticoid activity, whereas the Everse–de Fremery test assayed mineralocorticoid activity (Grundy et al. 1952, Tait et al. 2004). In fact, it is abundantly obvious that the two assays are fundamentally identical with each other and with the Vögtli method. As a curious addendum, it is also interesting to note that when work started on the purification of the glucocorticoid receptor (GR), for practical reasons DOC was used as the affinity ligand of choice (Failla et al. 1975).

**Figure 2** Data extracted from the papers of Ingle (1940a,b) (his Figs 1 (unoperated controls) and 3 (adrenalectomised animals)), and Vögtli (1943a,b) (his Table 1) and replotted. Adrenalectomised rats were treated for 7 days with 2 mg DOC (Ingle), or after 4–6 days with 1 mg DOCA (Vögtli), and then tested in the loaded gastrocnemius muscle contraction assay. The preparations were stimulated three times per second for 12 h (intact and adrenalectomised controls) or until the preparation failed (adrenalectomised and steroid treated) (Ingle), or for 8 h (Vögtli). The original data points were listed in a table (Vögtli) or individually plotted, with a mean value shown (Ingle). This figure shows mean ± s.d. (*n* numbers in columns), calculated by the present author. In each data set, adrenalectomised values are significantly lower than either unoperated or adrenalectomised and treated, *P*<0.0001. For clarity, limited data sets are shown here, both Ingle & Vögtli gave more, including comparison with cortisone or cortisone acetate (Ingle) or the adrenal extract cortin (Vögtli). Ingle also showed clear dose dependency of the DOC and DOCA effect. In his experiments, only cortisone acetate at 2 mg gave values higher than those obtained with DOC. The *y*-axis units are of the original authors. There is an error in Ingle’s *y*-axis, and the highest values here are, in reality, effectively up to 20,000 revolutions of his recorder, not 0–2 revolutions as the axis label suggests, which would give rise to an unfeasibly (and unmeasurably) low value for work performed. Ingle equates 1 revolution to 400 g cm work. This would give maximum values of about 60 m kg in Vögtli’s units. While this is at least five- to six-fold greater than Vögtli achieved (note Vögtli’s values are per 100 g BW: both authors used animals of about 150 g BW), it at least is of the same order of magnitude as Vögtli’s and the differences could perhaps be accounted for by differences in the equipment/technique, or the longer sampling periods used by Ingle. In any case, the strikingly similar data in the two figures here strongly indicate that the two authors measured the same thing. Vögtli interpreted his data to support the view that DOCA could completely restore the work capacity of the gastrocnemius muscle of the adrenalectomised rat. His conclusions were vehemently opposed by Ingle (1945) (see text).

**Mineralocorticoid activity**

Though the definition of mineralocorticoid activity is more limited than that of glucocorticoid activity, it somewhat surprisingly lacks universality. The one constant feature in any definition is sodium retention; however, this has often been coupled to regulation of other ions. To Selye & Jensen (1946), who introduced the term, mineralocorticoid activity, exemplified by DOC, meant ‘causing sodium retention and the restoration of the subnormal blood sodium and blood chloride levels in adrenalectomised animals’. The inclusion of chloride in this definition or in any bioassay is rare, though in fact highly relevant, since the restoration of blood volume requires salt, not just the cation (Crabbe 1992).

More common is the coupling of sodium with potassium ions. It is possible to speculate on the origins of this approach. At the time when the biological actions of the corticosteroids was being explored, there was a great interest in sodium–potassium ‘exchange’ and the mechanisms by which cell membrane polarisation was achieved. This culminated in the characterisation of the sodium/potassium ATPase of the basolateral plasma membrane (Skou 1957, 2003). Accordingly, it must have seemed reasonable to suppose that any action on sodium transport would be accompanied by a
coupled transport of potassium in the opposite direction and that the \( \text{Na}^+/\text{K}^+ \) ratio in urine would be a highly appropriate index to use. It may even have been supposed that mineralocorticoids would act directly on this ATPase-linked pump. But even at the time of Skou’s studies, it was known that there was additional sodium permeability at the apical rather than the basolateral cell membrane and that this was not linked to ‘exchange’ with potassium ions, except indirectly. We now know that this apical site, the epithelial sodium channel, is a primary target for aldosterone (Fig. 1) (Crabbe 1963, Kellenberger & Schild 2002, Bhargava et al. 2004, Naray–Fejes-Toth et al. 2004). So it is to be expected that mineralocorticoid action measured as sodium retention (or better, salt retention) may not be equivalent to mineralocorticoid action measured as changes in urinary \( \text{Na}^+/\text{K}^+ \) ratio. Indeed, it is not. It is because of this that the differences of opinion about the relative mineralocorticoid potencies of different steroids arise.

In their work on the isolation and characterisation of aldosterone, Tait et al. (1952) used a unique version of the urinary sodium/potassium assay, exploiting the newly developed algorithms for analysis of Na\(^{24}\) and K\(^{42}\) (Tait & Williams 1952). In their method, after administration of the isotopes, the urinary Na\(^{24}/\text{K}^{42}\) ratio was assayed over a 2 h period in immature adrenalectomised rats. This method gave emphatic, and seemingly definitive, quantitative differences in mineralocorticoid activities of different steroids, which were comparable with other published results. Thus, DOCA was 20 times as active as cortisone in the isotope ratio method, consistent with the values obtained by others in the Everse–de Fremery muscle work test, the Cartland and Kuizinga rat survival test, sodium retention in adrenalectomised dogs and in treatment of Addisonian patients (Grundy et al. 1952). It was also similar to the relative effectiveness of these two steroids in regulating blood urea in dogs, data that were not referenced, and not commented on, but that do not seem consistent with mineralocorticoid actions.

This method, which has not been used by other groups since, seems to have one significant disadvantage. The assumption is that the infused isotopes penetrate and are in equilibrium with the endogenous pools of these cations. The clarity of the results suggests that this is a reasonable assumption, although no evidence was provided. The use of a ratio of the excreted nuclides could be seen as a way of reducing some of the inevitable variables in isotope absorption and equilibration, by assuming that administered potassium and sodium are absorbed similarly. However, this limits the applicability of the method, and individual values for the two electrolytes are not available, a disadvantage in view of the discussion above. A non-isotopic assay based on the ratio of excreted \( \text{Na}^+/\text{K}^+ \) in adrenalectomised rats was developed by Kagawa (1960).

In their early correspondence with Tadeusz Reichstein, the Tait group revealed that by using the isotope ratio method, electrocortin (which was only called aldosterone when its structure was finally determined) was \( 80 \pm 10 \) times as active as DOCA (Tait & Tait 1998). Subsequent assays, using synthesised aldosterone, have given a wide range of values (Table 1).

At first, it seemed unlikely that \( \text{Na}^+/\text{K}^+ \) ratios might be misleading in ascribing greater activity to aldosterone than to DOCA – Genest (1955) found the same relative activities of aldosterone and DOCA for both \( \text{Na}^+ \) and \( \text{K}^+ \) excretion, giving significantly higher activity for aldosterone. In contrast,

<p>| Table 1 Mineralocorticoid activities of aldosterone and deoxycorticosterone acetate (DOCA) |</p>
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<th>Species</th>
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<th>Aldosterone value</th>
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<td>1</td>
<td>Urinary Na(^{24}/\text{K}^{42}) ratio</td>
<td>80 (electrocortin)</td>
<td>1</td>
<td>Tait et al. (1952)</td>
</tr>
<tr>
<td>1</td>
<td>Urinary Na(^{24}/\text{K}^{42}) ratio</td>
<td>120 (electrocortin)</td>
<td>1</td>
<td>Speirs et al. (1954)</td>
</tr>
<tr>
<td>1</td>
<td>Urinary Na(^{24}/\text{K}^{42}) ratio</td>
<td>25–50</td>
<td>1</td>
<td>Simpson et al. (1954)</td>
</tr>
<tr>
<td>2</td>
<td>Urinary Na(^{+}/\text{K}^{+})</td>
<td>&gt; 10</td>
<td>1</td>
<td>Prunity et al. (1954)</td>
</tr>
<tr>
<td>3</td>
<td>Maintenance</td>
<td>25–30</td>
<td>1</td>
<td>Gross &amp; Gysel (1954)</td>
</tr>
<tr>
<td>3</td>
<td>Maintenance</td>
<td>12–25</td>
<td>1 (DOCA)</td>
<td>Swingle &amp; Kleinberg (1955)</td>
</tr>
<tr>
<td>1</td>
<td>( \text{Na}^+ ) retention</td>
<td>100</td>
<td>8</td>
<td>Genest (1955)</td>
</tr>
<tr>
<td>1</td>
<td>( \text{K}^+ ) excretion</td>
<td>100</td>
<td>10</td>
<td>Agarwal (1994)</td>
</tr>
<tr>
<td>1</td>
<td>( \text{Na}^+/\text{K}^+ )</td>
<td>100</td>
<td>17</td>
<td>Uete &amp; Venning (1962)</td>
</tr>
<tr>
<td>1</td>
<td>( \text{Na}^+ ) decrease</td>
<td>0·191 mequiv/6 h*</td>
<td>0·103 mequiv/6 h*</td>
<td>Campen et al. (1983)</td>
</tr>
<tr>
<td>1</td>
<td>( \text{K}^+ ) increase</td>
<td>0·055 mequiv/6 h*</td>
<td>0·005 mequiv/6 h*</td>
<td>Galigniana et al. (2004)</td>
</tr>
<tr>
<td>1</td>
<td>( \text{Na}^+ ) retention</td>
<td>0·1–0·3 ( \mu \text{g}/100 \text{ g BW} )</td>
<td>10 ( \mu \text{g}/100 \text{ g BW} )</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>( \text{Na}^+ ) excretion</td>
<td>0·1–0·3 ( \mu \text{g}/100 \text{ g BW} )</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Agarwhal reported that the two steroids gave identical responses in Na\(^+\)/K\(^+\) values. It was Uete & Venning (1962) who first showed that compared with aldosterone DOCA activities were similar for sodium, but that DOCA was much less effective on potassium excretion. In contrast, cortisol has a much greater kaliuretic effect than aldosterone (Crabbe 1992).

This concept of differences between Na\(^+\) and K\(^+\) effects was developed by Campen et al. (1983), who distinguished between glucocorticoid- and mineralocorticoid-induced kaliuresis. In adrenalectomised animals treated subcutaneously at time 0 and 2.5 h, urinary electrolytes were estimated in a 2.5–5 h collection period and gave a threshold effect on Na\(^+\) at about a dose of DOC of 1 µg/100 g body weight, about tenfold more than that for aldosterone. However, the threshold effect of aldosterone on K\(^+\) occurred at about the same dose as required for Na\(^+\) retention, at about 0.1–0.3 µg, whereas for DOC it was 100 µg/100 g, remarkably different from the threshold for sodium. The response to aldosterone appeared to be biphasic, with a further increase in K\(^+\) excretion at higher doses, which they attributed to the ‘glucocorticoid’ (non-spironolactone inhibitable)-induced kaliuresis. Dexamethasone induced both Na\(^+\) excretion (inhibitory at high doses) and K\(^+\) excretion, whereas 9α-fluorocortisol had no consistent effect on Na\(^+\) but increased K\(^+\) excretion. There are interesting interactions between the steroids, for example aldosterone with dexamethasone gave less sodium excretion than with aldosterone alone, but greater kaliuresis than with either steroid alone. The differences between aldosterone and DOC in their relative effects on Na\(^+\) and K\(^+\) not unexpectedly give, as usually observed by others, the marked differences in their actions as measured by Na\(^+\)/K\(^+\) ratios. Indeed, these authors point out that the use of Na\(^+\)/K\(^+\) ratios can be highly misleading because, by this method, both dexamethasone and 9α-fluorocortisol might appear to be good mineralocorticoids. Their actions on the electrolyte ratio are, however, attributable to their disproportionate effects on potassium excretion.

The relatively poor activity of DOC on kaliuresis was adduced by Campen et al. (1983) as evidence that DOC is a partial glucocorticoid agonist. Whether or not they were right to ascribe its sodium effects to mineralocorticoid receptor (MR) activation alone, and potassium effects to both GR and MR mediation, the relatively poor effect of DOC on K\(^+\) relative to Na\(^+\) does not seem to be adequately accounted for. Why then, if there no MR-mediated kaliuretic effect of DOC? There is much still to be learned about the mechanisms underlying discriminatory actions of steroids that act only through limited types of receptor.

The essential key role of assay choice, and its interpretation, is clearly illustrated by the work of Galigniana et al. (2004). Here, because the Na\(^+\) excretion/steroid concentration–response curves are biphasic, and Na\(^+\) excretion nearly always rises at very high steroid concentrations, the authors used another index. This is a which represents the second-order coefficient of the polynomial in the relation: 

\[ y = ax^2 + bx + c, \]

which describes the dose–response curves. In this measure, DOC is actually more potent than aldosterone.

As an addendum, it is interesting to note that the actions even of aldosterone on potassium excretion are not necessarily physiological, and though its role may once have been considered crucial, other mechanisms for potassium regulation must now be considered relevant (Rabinowitz 1996, Greenlee et al. 2009).

### Effects of DOC in man

There is little in the literature to suggest that DOC should actually be considered to be a hormone, if that implies secretion is significantly and specifically regulated in response to physiological demand. Normally, secretion rates of DOC are low (e.g. 0.089 mg/day compared with 3-3 mg corticosterone/day in young men (Romanoff & Baxter 1975) or 100–200 µg/day for aldosterone (Katzung 2004, Goodman et al. 2007); other authors give higher values for DOC, e.g. 394.7 µg/day (Harris et al. 1967), and its plasma concentration is typically 200–400 pmol/l (Brown & Strott 1971, Oddie et al. 1972, Schonoshfer et al. 1975). Its secretion may be enhanced by ACTH and by angiotensin II and potassium ions, suggesting both a zona fasciculata and a zona glomerulosa origin (Brown et al. 1972b), and it is also inevitably enhanced in conditions in which 11β-hydroxylase (CYP11B1) has reduced activity. These may be as a result of defects in the enzyme, resulting in the relatively rare salt-retaining form of congenital adrenal hyperplasia (CAH), or as a result of treatment of adrenocortical excess with an 11β-hydroxylase inhibitor such as metyrapone or etomidate (Harris et al. 1967, Weber et al. 1993). CAH too gives relatively little clear indication of a multiplicity of DOC actions, although there may be various reasons for this. An obvious point is that the symptoms in newborn infants, of hypertension and masculinisation, are so severe as to mask any more subtle changes, and to require immediate correction. The second is that the abnormalities of circulating steroid profiles are of course not confined to excess DOC. Quite apart from the lack of cortisol (or corticosterone), and androgen excess, there is a further complication in that increased DOC secretion is accompanied by increased 11-deoxycortisol, a steroid whose actions have been relatively little studied. Indeed, 11-deoxycortisol is the major steroid overproduced in this syndrome (Caulfield et al. 2002).

Like DOC, 11-deoxycortisol also binds to both GR and MR (Hellal-Levy et al. 1999) and has roughly the same activity as DOC in a rat colon sodium transport assay (Grotjohann et al. 1999), an effect that might be mediated by either receptor type (Fuller et al. 2000, Zeissig et al. 2006). Despite that, the hypertension and hypokalemia associated with 11β-hydroxylase deficiency is commonly attributed to DOC alone (New 2002, Tonetto-Fernandes et al. 2006).
The use of metyrapone adds little more information, though there has been some speculation. Inhibition of cortisol secretion in depressed patients has been a clinical objective, but it has also been noted that since depression is normally associated with low circulating levels of DOC (and deoxycorticisol), the ameliorative effects of metyrapone may in part be attributable to the increased levels of 11-deoxysteroids, as well as to decreased 11β-hydroxysteroids such as cortisol or corticosterone (Raven et al. 1996).

It should be noted nevertheless that there is some evidence that DOC may be produced within tissues, such as the brain or ovary, where it has the potential to promote paracrine actions not reflected in its circulating concentrations (see below).

Other actions, and DOC in other species

In vitro at least, the adrenal homologues in potentially all non-mammalian vertebrate species that have been examined have the capacity to produce DOC, since all possess a functional 21-hydroxylase (Vinson et al. 1979, 1992). However, proof of its presence in circulating plasma has only rarely been shown: for *Rana catesbiana*, *Tilapia aurea* and *Oncohyonus mykiss* and the lamprey see Taylor et al. (1972a), Katz & Eckstein (1974) and Campbell et al. (1980). It is particularly interesting that DOC is present in the teleost fish, which, conversely, generally do not make aldosterone (but see Whitehouse & Vinson (1975)), and for this reason, it has been speculated that DOC, which binds strongly to the fish MR, may be their primary mineralocorticoid (Sturm et al. 2005). This is controversial, the teleost 17-hydroxylase is fairly non-specific (Sandor et al. 1966, Sangalang et al. 1972), and 17-deoxysteroids, including DOC, are not in abundance: most authors believe that cortisol is the major teleost mineralocorticoid as well as glucocorticoid (Wendelaar Bonga 1997, McCormick et al. 2008). On the other hand, DOC together with 11-deoxycorticisol is apparently present, whereas corticosterone, aldosterone and cortisol are absent, in the plasma of the lamprey, and Close et al. (2010) conclude that 11-deoxycorticisol is the major corticosteroid in this species, with both glucocorticoid and mineralocorticoid actions. Though the concept has been challenged (Thornton & Carroll 2011), these findings are undoubtedly interesting and provocative. There is a clear difficulty in interpreting such data from any marine fish, including the Agnatha. Because they live exclusively in a hypertonic environment, there is no clear requirement for any mineralocorticoid activity at all – indeed sodium elimination would seem to be a major problem in electrolyte homeostasis. In marine teleosts, this is carried out primarily at the gill, under the regulation of cortisol (Foskett et al. 1983, Marsiglione et al. 2000, Madsen et al. 2007). Conventional distinction between mineralocorticoids and glucocorticoids, which presents difficulties enough in a mammalian context (Vinson 2009), should obviously be used with great care with regard to exclusively marine animals.

Hypertension is a widely recognised effect of DOC, as noted above. This effect of DOC has been used in experimental induction of hypertension in animals, for example in regenerating enucleated adrenal in the rat. Here, the regenerating gland is relatively deficient in 11β-hydroxylase, so corticosterone production is depressed whereas DOC becomes elevated (Brown et al. 1972a, Holzbaier et al. 1972): 19-nor-DOC and other DOC derivatives may also be increased and implicated in the development of hypertension in these animals (Dale et al. 1982, Gomez-Sanchez et al. 1983). Additionally, the salt-fed DOCA-treated rat is a standard model for hypertension, and although this could be assumed to be an adjunct to its mineralocorticoid action, the effect is complex and central, and other actions are implicated (Schenk & McNeill 1992, Pinto et al. 1998, Yemane et al. 2010) – as indeed is also the case for aldosterone (Xue et al. 2011). The salt-DOCA rat model of hypertension must be the most widely used application of DOCA treatment – a search of the key words in Web of Science produces a count of some 1700 relevant papers.

However, DOC is still more versatile. Perhaps because of its close structural resemblance to progesterone, it has similar actions in some systems (van Heuverswyn et al. 1939) and gives, for example, decidual responses in ovariectomised guinea pig uteri (Blaha & Leavitt 1978). It also induces relaxation of the symphysis pubis in these animals, apparently because, like progesterone, it induces release of relaxin (Hall 1960). Such actions have not been reported for cortisol or corticosterone. It is possible that this has physiological implications: DOC (but not cortisol) has been shown to be secreted by the human ovary (Nahoul et al. 1988), and there is

![Figure 3 Neuroactive steroids. THDOC and 3α,5β-tetrahydroDOC are derived from DOC in the brain, where they exert anxiolytic and other actions. The progesterone derivatives have similar actions.](https://example.com/figure3.png)
a relatively high level of circulating DOC sulphate in pregnant women, which originates in the placenta by transformation of fetal pregnenolone disulphate (Corsan et al. 1997). Formation of DOC is also suggested in ovarian follicles in humans in stimulated cycles, and in polycystic ovary disease (Dehennin et al. 1987, Ito et al. 1987), in the ovaries of macaques (Fru et al. 2006) and in the testes or ovaries of various fish species (Milla et al. 2009). DOC (or DOCA) stimulates oocyte maturation in catfish and other fish species (Sundararaj & Goswami 1971, Milla et al. 2009), Rana pipiens (Schuetz 1972) and hens (Etches & Cunningham 1976) and has progestin-like activities in inducing gonadotrophin surges in hens (Wilson & Sharp 1976), in immature rats (Brann et al. 1990) and in oestrogen-primed ovariectomised mice (Mahesh & Brann 1992). It can stimulate premature hatching embryos in the teleost Oryzias latipes (Cloud 1981).

Additionally, as DOC is the precursor of the neuroactive steroids 3α,5α-tetrahydro-DOC (THDOC) and 3α,5β-tetrahydro-DOC, it has the potential for many more profound actions. The neurosteroids, which also include 3α,5α-tetrahydroprogesterone (THP, allopregnanolone), 3α,5β-tetrahydroprogesterone (Fig. 3) and others, may act primarily through allosteric sites on the GABA A receptor (Paul & Purdy 1992, Rupprecht 2003, Morrow 2007). In particular, THDOC and THP (Fig. 3) have GABA A receptor-positive modulatory effects and produce inhibitory neurobehavioural effects, including anxiolytic, anticonvulsant and sedative actions (Biggio et al. 2007, Morrow 2007) and may mediate some of the actions of ethanol (Biggio et al. 2007, Boyd et al. 2010, Kaufman et al. 2010). THDOC and allopregnanolone also have actions on the hypothalamus pituitary-adrenal axis and thereby on the effects of stress (Girdler & Klatzkin 2007). Such positive GABA A potentiation may also indirectly affect reproduction via the pituitary (Henderson 2007). Negative GABA A regulators include the sulphated derivatives of pregnenolone and DHEA as well as the 3α,5α- and 3α,5β-reduced metabolites of cortisol (Morrow 2007). Other neuroactive steroids (such as pregnenolone, progesterone, allopregnanolone and dehydroepiandrosterone) are attributed with actions in central nervous system (CNS) development (Mellon 2007). The debate about the origin of the DOC precursor for its neuroactive THDOC metabolite continues, but although steroidogenesis, and the production of the steroid regulators of GABA has been reported in the CNS (Morrow 2007), it is still likely that the adrenal is an important source (Boyd et al. 2010, Kaufman et al. 2010).

While we are accustomed to think of sodium homeostasis in terms of kidney, or epithelial, function, other factors may come into play in the whole animal. In part, these may be behavioural, and in studies on sodium appetite in Balb/c mice (Blair-West et al. 1995), DOC was clearly significantly more potent than either aldosterone or corticosterone.

Ectopic production of DOC has also been postulated in the thymus, where it has a potential role in thymocyte selection (Vacchio et al. 1994).

The wide variety of DOC’s actions presumably accounts for its utilisation as an insect defence mechanism, like certain

<table>
<thead>
<tr>
<th>Function</th>
<th>DOC</th>
<th>Aldosterone</th>
<th>Cortisol, cortisone, or corticosterone</th>
<th>Example references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor binding/activation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Bledsoe (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hall-Lewy et al. (1999)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Campen et al. (1983)</td>
</tr>
<tr>
<td>Sodium retention</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Montigel &amp; Verzar (1943a,b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Voglli (1943b) and Sass-Kortsak et al. (1949)</td>
</tr>
<tr>
<td>Potassium elimination</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Verzar &amp; Wang (1950)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ingle (1950)</td>
</tr>
<tr>
<td>Glycogen phosphorylation</td>
<td>+</td>
<td></td>
<td>+</td>
<td>Cheng &amp; Sayers (1949)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Voglli (1943a,b)</td>
</tr>
<tr>
<td>Glycogen deposition</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Olson et al. (1944a,b) and Conway &amp; Hingerty (1953)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thornt (1938) and Conway &amp; Hingerty (1953)</td>
</tr>
<tr>
<td>Glycogenolysis</td>
<td>+</td>
<td></td>
<td></td>
<td>Verzar (1950)</td>
</tr>
<tr>
<td>Induce insulin resistance</td>
<td>-</td>
<td></td>
<td></td>
<td>Cheng &amp; Sayers (1949)</td>
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<tr>
<td>Induce insulin sensitivity</td>
<td>+</td>
<td></td>
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<td>Vogelli (1943a,b)</td>
</tr>
<tr>
<td>Muscle work</td>
<td>+</td>
<td></td>
<td></td>
<td>Olson et al. (1944a,b) and Conway &amp; Hingerty (1953)</td>
</tr>
<tr>
<td>Anabolic (BW gain)</td>
<td>+</td>
<td></td>
<td></td>
<td>Thornt (1938) and Conway &amp; Hingerty (1953)</td>
</tr>
<tr>
<td>Survival following adrenalectomy</td>
<td>+</td>
<td></td>
<td></td>
<td>Verzar &amp; Wang (1950)</td>
</tr>
<tr>
<td>Treatment of Addisonian patients</td>
<td>+</td>
<td></td>
<td></td>
<td>Thornt (1939a,b)</td>
</tr>
<tr>
<td>Sodium appetite (mice)</td>
<td>+</td>
<td></td>
<td></td>
<td>Blair-West et al. (1995)</td>
</tr>
<tr>
<td>Actions on reproductive tract in rodents and non-mammalian spp</td>
<td>+</td>
<td></td>
<td></td>
<td>van Heuverswyn et al. (1939), Blaha &amp; Leavitt (1978) and Milla et al. (2009)</td>
</tr>
</tbody>
</table>
other steroid types. DOC in particular is synthesised and stored in various beetle species, including Dytiscus marginalis, Aelius sulcatus and Agabus bipustulatus (Schildknecht 1970), and as much as 1 mg has been reported to be present in Cybister limbatus prothoracic glands (Morgan 2004). It has no actions in the insect itself, but can be expected to have a devastating effect on fish, and presumably other predators (Schildknecht 1970).

Further unconfirmed reports suggest other functions, for example high concentrations of DOC promote sodium and potassium exchange in Neurospora crassa (Lester & Hechter 1959), and inhibition of growth in this species and in Penicillium puberulum, a property shared with oestradiol-17β (Lester & Hechter 1961). This was taken to suggest a direct interaction of steroid with intracellular components involved in the transport mechanisms, a view that accords with other ideas prevalent at the time, for example direct DOC interaction with ATP (White 1960, Dixon et al. 1964), but this was before the concept of nuclear receptors had begun to be developed.

Conclusions

It must be clear that DOC is a steroid like no other, with a unique set of activities, at least under experimental conditions (Table 2). These range from electrolyte exchange and metabolic actions to reproduction, behaviour and even defensive properties. And yet, its secretion, in most mammals, is insufficient to suggest a homeostatic or metabolic role. Not only is it secreted in low amounts, its pattern of secretion has not suggested that it is anything but an intermediate in the pathway to the major secreted adrenal products, cortisol, corticosterone and aldosterone – at least in the adrenal: paracrine DOC production cannot be excluded (see above).

So perhaps the reason it is not secreted in large amounts is precisely because its wide range of activities would make that undesirable. Instead, by the addition of an oxygen function at C-11, the major steroids become more specific and their functions limited: not only does, say, cortisol have a different pattern of secretion has not suggested that it is anything but an intermediate in the pathway to the major secreted adrenal products, cortisol, corticosterone and aldosterone – at least in the adrenal: paracrine DOC production cannot be excluded (see above).

But perhaps the most important conclusion lies in the understanding of steroid structure–function relationships. We do not have to try to explain why DOC should reportedly be a weak mineralocorticoid, but the addition of an oxygen function at C-11, the major steroids become more specific and their functions limited: not only does, say, cortisol have a different pattern of secretion has not suggested that it is anything but an intermediate in the pathway to the major secreted adrenal products, cortisol, corticosterone and aldosterone – at least in the adrenal: paracrine DOC production cannot be excluded (see above).

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