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SPECIES VARIATION IN STEROID BIOSYNTHETIC PATHWAYS: THE FORMATION OF CORTISOL IN HAMSTER ADRENAL TISSUE *IN VITRO*

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SUMMARY

Kinetic studies on the biosynthesis of cortisol, cortisone and corticosterone from [^{3}H]pregnenolone and [^{14}C] progesterone by hamster adrenal tissue show that the two precursors were handled similarly. There were no significant differences between the yield–time curves for tritiated and [^{14}C]-labelled intermediates and products as judged by inspection, and no singly labelled products were isolated. An early maximum in the formation of [^{3}H]progesterone suggests that this compound is the first intermediate formed from pregnenolone during the synthesis of both 17α-hydroxy- and 17-deoxycorticosteroids. This contrasts with results obtained by similar methods using human, sheep and rabbit adrenal tissue, in which 17α-hydroxypregnenolone appeared to be much more important than progesterone. There may be adaptive or evolutionary significance in this pronounced species variation.

INTRODUCTION

CONSIDERABLE evidence now suggests that in the formation of cortisol by human, sheep, and rabbit adrenal tissue incubated *in vitro*, pregnenolone is transformed not into progesterone, as originally thought, but into 17α-hydroxypregnenolone, and thence to 17α-hydroxyprogesterone, 11-deoxycortisol and cortisol[1–6].

Consequently the Δ^5 pathway is now generally accepted as a major route of cortisol biosynthesis. In the case of corticosterone biosynthesis, however, there seems to be a degree of species variation in the nature of the preferred pathway, and whereas in many species no distinction could be made between the roles of [^{3}H]pregnenolone and [^{14}C]progesterone as substrates[7–11] for corticosterone formation, in sheep experiments the formation of 21-hydroxypregnenolone suggested an alternative route which bypasses progesterone[12].

We report here some results obtained with hamster adrenal tissue which suggest that similar species differences may occur in the pathways involved in the formation of cortisol.

MATERIALS AND METHODS

In a typical experiment, hamster adrenal tissue (100 mg) was incubated with 5·0 μCi [16-^{3}H]pregnenolone (S.A. 3·6 mCi/mg) and 0·1 μCi [4-^{14}C]progesterone (S.A. 69 μCi/mg) in 10 ml Ringer solution at 37°C under an atmosphere of 95%O_2, 5%CO_2. One ml samples of the medium were removed at selected time intervals, and these were replaced by similar volumes of fresh Ringer solution without added precursors. Similar incubations were performed using [^{14}C] 17α-hydroxyprogesterone (0·2 μCi; S.A. 113 μCi/mg). Further experiments to facilitate identification of the products were performed using larger amounts of tissue (up to 1000 mg) and 0·4 μCi [4-^{14}C]progesterone with a single incubation

time of 2 h. All samples of incubation media were separately extracted with ethyl acetate. Fractionation of steroids was performed in various chromatographic systems (Table 1). All extracts were originally fractionated in System 1. Material less polar than 11-deoxycortisol was eluted, treated with acetic anhydride/pyridine, and rechromatographed in System 2. Yields of products were estimated on aliquots at this stage, and further samples were then taken through additional procedures to check the identity of the products (Tables 2, 3). Yields of products were corrected for dilution and plotted against time (Figs. 1–3).

Table 1. Chromatographic systems used in isolation and characterisation of hamster adrenal products. In each system the support material was Whatman No. 1 paper.

System	Solvents	Average duration of run (h)
1	Light petroleum: 36% aq. t-butanol	3
2	Light petroleum: 75% aq. methanol	1·5
3	Cyclohexane/benzene (2/1): 80% aq. methanol	2·5
4	Toluene: 70% aq. methanol	2·5
5	Isooctane: 80% aq. t-butanol/methanol (1/1)	3

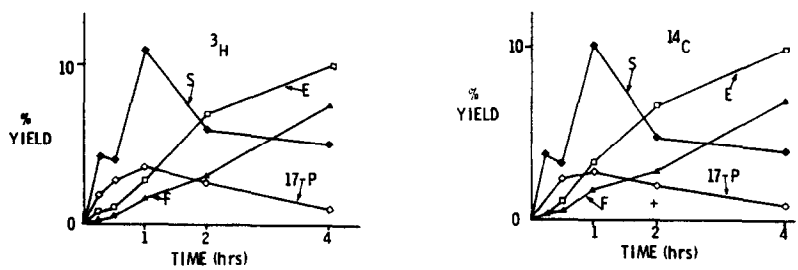

Fig. 1. Yield–time curves for 17α-hydroxylated compounds isolated during incubation of hamster adrenal tissue with [16-^{3}H]pregnenolone and [4-^{14}C]progesterone. 17-P = 17α-hydroxyprogesterone; S = 11-deoxycortisol; F = cortisol; E = cortisone.

RESULTS

The major radioactive products isolated in [^{3}H]pregnenolone/[^{14}C]progesterone incubations were identified (Tables 2, 3) as cortisol, cortisone, 11-dehydrocorticosterone, corticosterone, 11-deoxycortisol, 17α-hydroxyprogesterone, deoxycorticosterone, and progesterone. All these were doubly labelled. Unchanged [^{3}H]pregnenolone was also recovered. The product yield time curves for both precursors are shown in Figs. 1–2. No other major products were detectable, and no singly labelled product was isolated. In particular, [^{3}H]17α-hydroxypregnenolone and [^{3}H]21-hydroxypregnenolone were sought but could not be detected. Yield time curves for products from [^{14}C]17α-hydroxyprogesterone are shown in Fig. 3.

DISCUSSION

In earlier experiments of the type described here it was found that certain

Table 2. Procedures for identification of hamster adrenal products

Compound	Procedure	R_f identical with:	System
Cortisol	Extraction	Cortisol	1, 4
	Oxidation*	11β-Hydroxyandros-tenedione	2
	Acetylation†	Cortisol acetate	3
Cortisone	Extraction	Cortisone	1, 4
	Oxidation	11-Oxoandros-tenedione	2
	Acetylation	Cortisone acetate	3
Corticosterone	Extraction	Corticosterone	1, 2, 4
	Acetylation	Corticosterone acetate	3
11-Deoxycortisol	Extraction	11-Deoxycortisol	1, 2, 4
	Acetylation	11-Deoxycortisol acetate	3
	Oxidation	Androstenedione	2
11-Dehydrocorticos-terone	Extraction	11-Dehydrocorticos-terone	1, 2, 4
	Acetylation	11-Dehydrocorticos-terone acetate	3
Deoxycorticos-terone	Extraction	Deoxycorticosterone	2, 4, 5
	Acetylation	Deoxycorticos-terone acetate	2
17α-Hydroxy-progesterone	Extraction	17α-Hydroxy-progesterone	2, 4
	Attempted acetylation	17α-Hydroxy-progesterone	2, 4
Progesterone	Extraction	Progesterone	2, 4
	Attempted acetylation	Progesterone	2, 4

*Sodium bismuthate oxidation.
†Acetylation with acetic anhydride/pyridine.

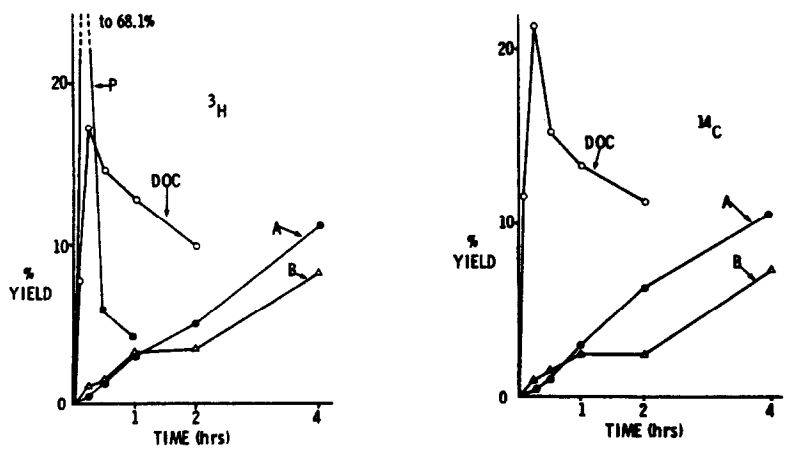

Fig. 2. Yield–time curves for 17-deoxy steroids isolated during incubation of hamster adrenal tissue with [16-^{3}H]pregnenolone and [4-^{14}C]progesterone, under the same conditions as for Fig. 1. P = progesterone; DOC = deoxycorticosterone; B = corticosterone; A = 11-dehydro-corticosterone.

Table 3. Recrystallisation of [^{14}C]-labelled hamster adrenal products (formed during incubation from [^{14}C]progesterone) with authentic [^{3}H]-labelled and unlabelled material. Solvents used were petroleum ether (b.p. 40–60°C), ether and ethyl acetate

	Crystallisation	^{3}H/^{14}C ratios
Cortisol	1	5·8
	2	6·1
	3	6·1
Cortisone	1	5·5
	2	4·8
	3	5·5
11-Deoxycortisol	1	4·4
	2	4·0
	3	4·1
11-Dehydrocorticosterone*	1	2·4
	2	2·9
	3	2·7
Deoxycorticosterone	1	9·1
	2	9·2
	3	8·7
17α-Hydroxyprogesterone	1	14·1
	2	14·0
	3	14·0

*Doubly-labelled material obtained from an incubation with [^{3}H]pregnenolone and [^{14}C]progesterone, crystallised with authentic 11-dehydrocorticosterone.

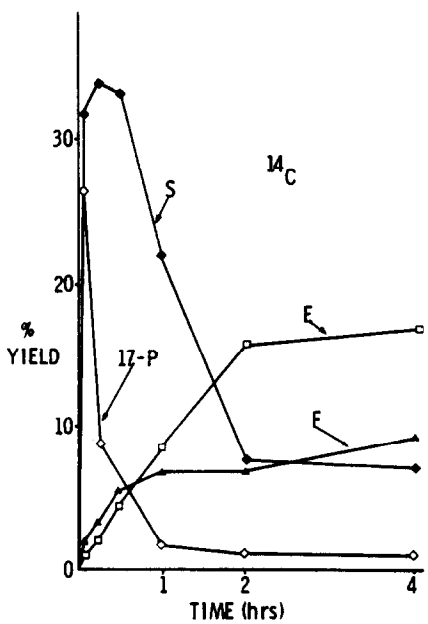

Fig. 3. Yield–time curves for products isolated during incubation of hamster adrenal tissue with [4-^{14}C]17α-hydroxyprogesterone; symbols as for Fig. 1.

conclusions could be reached by inspection of the product yield-time curves, although because of certain approximations inherent in the method the results do not lend themselves to systematic mathematical analysis (see Vinson and Whitehouse [13] for review).

In the first place, as a general rule, it may be noted that the order in which intermediates occur along a biosynthetic pathway is reflected by the sequential maxima in their incorporation of isotope. Secondly, where two differently labelled precursors are used simultaneously (as in the present experiments) and they occur along a single pathway, the sets of product yield time curves for the two labels may be remarkably similar. In contrast, where the two precursors are handled differently by the tissue, the two groups of yield time curves may be very different, and one or more singly labelled compounds may be isolated in large yield.

In the present experiments, the sets of ^{3}H and ^{14}C product yield time curves are remarkably similar in both the 17α-hydroxycorticoid and 17-deoxycorticoid series (Figs. 1 and 2). No singly labelled compounds were isolated, and the yields of the products from the two precursors are virtually identical. There is no lag between the timing of the ^{14}C and the ^{3}H maxima, which might be expected in view of the fact that in the conventional pathways, pregnenolone precedes progesterone. However, the conversion of pregnenolone to progesterone was obviously extremely rapid, as shown by the very large maximum in [^{3}H]progesterone which occured after only five minutes of incubation. Consequently it may be concluded that differences in the timing of maximal yields from pregnenolone and progesterone will be only slight, and not detectable at the time intervals arbitrarily chosen. In both tritium and ^{14}C, maxima in progesterone and deoxycorticosterone preceded those in corticosterone and 11-dehydrocorticosterone, while maxima in 17α-hydroxyprogesterone and 11-deoxycortisol preceded those in cortisol and cortisone (Figs. 1–3).

This pathway contrasts with those hitherto described for the biosynthesis of cortisol in human, sheep and rabbit adrenal preparations, in which 17α-hydroxypregnenolone was the first intermediate formed from pregnenolone. It seems likely that there is a degree of species variation in the preferred pathway for cortisol formation, as there is in the pathways by which other hormones are biosynthesised. Presumably there is some adaptive or evolutionary significance in this variation, and it is possible that mechanisms of control of hormone secretion show similar variability (Fig. 4).

The relationship between cortisol and corticosterone, and their companions, cortisone and 11-dehydrocortisone, is of further interest. As has already been shown, the 11-dehydrogenase system in adrenal cortex of the hamster incubated in Krebs–Ringer solution is extremely active. In incubations cortisone was always produced in greater amounts than cortisol although cortisol was the major constituent of the adrenal venous secretion [14]. It was shown that incubations of hamster adrenal tissue in hamster (or rat) blood gave results which approximated more closely to the *in vitro* condition, and it was concluded that incubation under these conditions affected the cortisol–cortisone equilibrium through interaction with adrenal enzymes [15].

In the present incubations, the role of cortisol as an intermediate in cortisone production is not apparent from their yield–time curves, in that there is no maximum in cortisol formation from either precursor which clearly precedes the

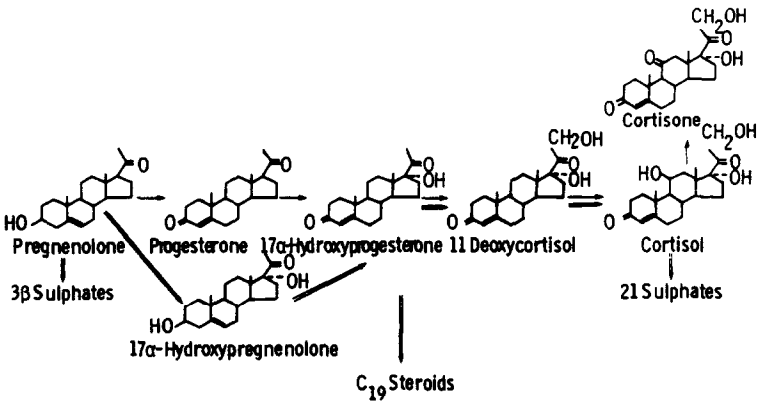

Fig. 4. Formation of 17α-hydroxycorticoids by adrenal tissue *in vitro*. → hamster; ⇒ man. cf. Ref. 13.

maximal formation of cortisone. Instead both products increase in yield throughout the incubation, and are present in proportions which remain approximately constant. This is also true of the relationship between corticosterone and 11-dehydrocorticosterone.

It is possible that the action of the dehydrogenase systems involved in the sequences cortisol → cortisone, and corticosterone → 11-dehydrocorticosterone are significantly reversible, and that the constant relative amounts of the compounds reflect the achievement of an equilibrium. There seems to be no precedent for this in other dynamic studies of steroid hormone biosynthesis *in vitro*. (cf. Ref. 13).

REFERENCES

1. I. Weliky and L. L. Engel: *J. biol. Chem.* **238** (1963) 1302.
2. B. J. Whitehouse and G. P. Vinson: *Steroids* **11** (1968) 245.
3. E. H. D. Cameron and K. Griffiths: *J. Endocr.* **41** (1968) 327.
4. N. Deshpande, V. Jensen, P. Carson, R. D. Bulbrook and T. W. Doouss: *J. Endocr.* **47** (1970) 231.
5. H. R. Fevold: *Biochemistry* **8** (1969) 3433.
6. A. B. M. Anderson, C. G. Pierrepoint, T. Jones, K. Griffiths and A. C. Turnbull: *J. Reprod. Fert.* **22** (1970) 99.
7. G. P. Vinson: *J. Endocr.* **34** (1966) 355.
8. B. J. Whitehouse and G. P. Vinson: *Gen. Comp. Endocr.* **9** (1967) 161.
9. S. W. C. Chan, G. P. Vinson and J. G. Phillips: *Gen. Comp. Endocr.* **12** (1969) 644.
10. D. P. Huang, G. P. Vinson and J. G. Phillips. *Gen. Comp. Endocr.* **12** (1969) 637.
11. S. T. H. Chan and B. R. Edwards: *J. Endocr.* **47** (1970) 183.
12. G. P. Vinson: *Gen. Comp. Endocr.* **9** (1967) 154.
13. G. P. Vinson and B. J. Whitehouse: In *Advances in Steroid Biochemistry and Pharmacology* (Edited by M. H. Briggs). Academic Press, London. Vol. 1 (1970) pp. 163–342.
14. B. J. Whitehouse, G. P. Vinson and P. A. Janssens: *J. Endocr.* **37** (1967) 139.
15. B. J. Whitehouse and G. P. Vinson: *J. Endocr.* **39** (1967) 117.