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THE EFFECT OF SYNTHETIC ACTH ON THE METABOLISM OF [4-^{14}C]-PROGESTERONE BY THE PREVIABLE HUMAN FETUS

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SUMMARY

Isolated human fetuses (16–18 weeks gestation) were perfused with [4-^{14}C]-progesterone in the presence or absence of synthetic ACTH. There was no significant difference in the amounts of unconjugated metabolites isolated from the adrenals and the perfusates in each case but in the conjugated extracts there was a significant increase in the percentage of extracted ^{14}C associated with the monosulphate fraction in the presence of ACTH. These increased sulphates were mainly accounted for by corticosterone and 11 deoxycorticosterone sulphates and, in addition, 11 deoxycortisol in the perfusates.

The results confirm that the metabolism of [4-^{14}C]-progesterone to corticosteroids in the mid pregnancy human fetal adrenals is stimulated by synthetic ACTH.

INTRODUCTION

It has been known for some time that during intrauterine life the fetal endocrine glands are under the control of the fetal pituitary and further, that the fetal pituitary appears to be active from an early stage in pregnancy. Fetal pituitary ACTH has been detected in the gland by the 10th week of gestation [1] and was considered by Lanman[2] to be the trophic hormone responsible for fetal adrenal hypertrophy.

More recently, interest in the effect of fetal ACTH on the production of fetal cortisol has been stimulated by the work of Liggins[3] on the fetal involvement in the events leading to the onset of parturition in the ewe and its application to the human. Liggins proposed the cascade phenomenon which suggests that initiating factors in the ewe may be an increase of ACTH causing stimulation of the adrenal cortex with the production of increased amounts of cortisol resulting in a series of events leading to myometrial contractions. This has resulted in interest in the application of this phenomenon to the onset of human parturition.

Indirect evidence of the effect of ACTH on the fetal adrenal has been demonstrated by the increase in maternal oestrogen excretion after injection of ACTH into the fetus [4] and Isherwood and Oakey[5] have provided biochemical evidence of increased steroid production by the human fetal adrenal in response to ACTH *in vitro*. Production of corticosteroids from progesterone by the fetal adrenal has been well established [6, 7]. It was therefore decided to investigate the effect of ACTH on the metabolism of progesterone in the fetal adrenal by a perfusion technique and to ascertain whether or not this trophic hormone could stimulate increased production of corticosteroids from progesterone by the human fetal adrenal at mid pregnancy.

MATERIALS AND METHODS

All chemicals used were of A.R. grade or were purified according to Fieser (1955).[8] Tritiated steroids were purchased from the Radiochemical Centre, Amersham and were purified by paper chromatography prior to use. Unlabelled steroids were purchased from Steraloids Ltd. Fetuses of 16–18 weeks gestational age were obtained at therapeutic termination of pregnancy by hysterotomy for socio-medical reasons.

Perfusion of Fetuses. Eight human fetuses (4♂ and 4♀) of 16–18 weeks gestational age were perfused by a modification of the method of Westin, Nyberg and Enhorning (1958) [9] as shown in Fig. 1 and described previously [10]. The umbilical venous cannula was attached via a needle containing an injection port to a drip set which contained group 'O' Rh. negative blood which had been adjusted to pH 7.4 using 4% (W/V) sodium bicarbonate solution and oxygenated by bubbling oxygen over a thin film of the blood. Progesterone was added to this blood to give a level of 24 μg/ml (the physiological level found in our laboratory in umbilical venous blood at 16–18 weeks). In 4 of the fetuses (2♂ and 2♀) this blood contained, in addition, 1.5 mg/100 ml of corticotrophin (Cortrosyn–Organon Ltd.) whilst in the other 4 fetuses (2♂ and 2♀) no synthetic ACTH was added. Each fetus was perfused with 100 μCi [4^{14}C]-progesterone. The radioactive steroid was injected via the injection port into the umbilical vein in a total of 1 ml. of ethanol–saline (1:1 v/v)—20% of the [4-^{14}C]-progesterone being added in each of 5 injections at ten minute intervals. The perfusions were continued at 37° for 2 h, the fetuses then removed, dissected and the fetal organs and the perfusates were placed immediately in a dry ice–acetone freezing mixture and stored at 20°C until processed.

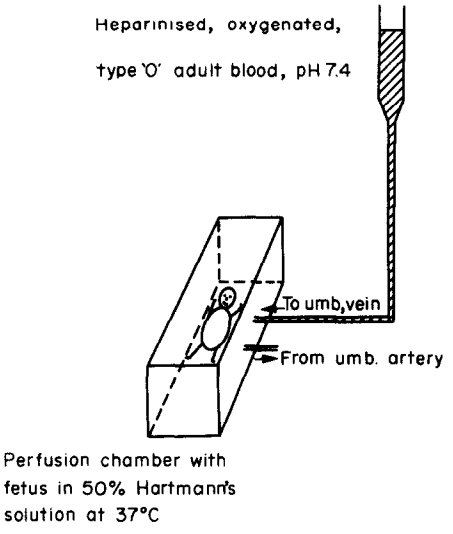

Fig. 1. Fetal perfusion apparatus.

Extraction. The fetal organs and perfusates were extracted with ethanol and 80% ethanol as described previously. [10]

Fractionation, characterisation and quantitation of metabolites. A mixture of ^{3}H labelled steroids containing all of the metabolites of interest, including 11 deoxycortisol, cortisol, cortisone, deoxycorticosterone and corticosterone was added to the dried extracts to allow subsequent quantitation and characterisation of any such steroid isolated bearing a ^{14}C label. As shown in Fig. 2, these dried extracts were partitioned between diethyl ether and water to separate the unconjugated and conjugated steroids.

Figure 3 shows a flow sheet of the methods used for fractionation, characterisation and quantitation of steroids from the unconjugated extract. The extracts were subjected to 2 dimensional t.l.c. in the systems—chloroform–methanol–water (94:6:0.5 by vol.) and cyclohexane–ethylacetate (1:1 v/v) [11] and each individual steroid eluted was subjected to sequential derivative formation—20 β reduction, acetylation and oxime formation. Each derivative was purified by t.l.c. Radioactivity present in aliquots of each chromatographic eluate was determined and the ^{3}H:^{14}C ratio calculated for each. Steroids were taken to be characterised when the final 3 ratios were constant ($\pm 5\%$ of the mean ratio). [12] Quantitation by double isotope dilution principles was based on the d.p.m. ^{3}H and ^{14}C present in the final derivative.

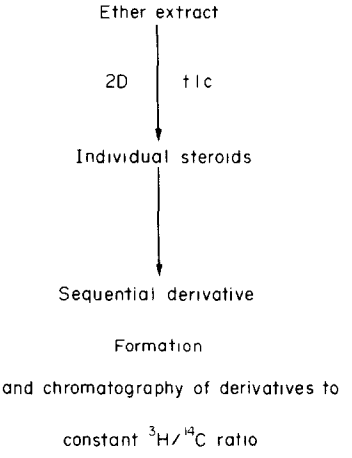

Fig. 3. Fraction of unconjugated steroids.

Figures 4 and 5 depict a flow sheet of the methods used for fractionation, characterisation and quantitation of the conjugated steroids. Mixtures of marker ^{3}H standards of polar free steroid, steroid mono and disulphates and steroid glucuronide were added to the aqueous extracts from the ether–water partitions. These extracts were then subjected to column chromatography on LH20 sephadex columns (50 cm × 1 cm) to separate the metabolites into free, glucuronide, monosulphate and disulphate zones.

Figure 6 shows a typical elution pattern of steroid zones following chromatography of an aqueous

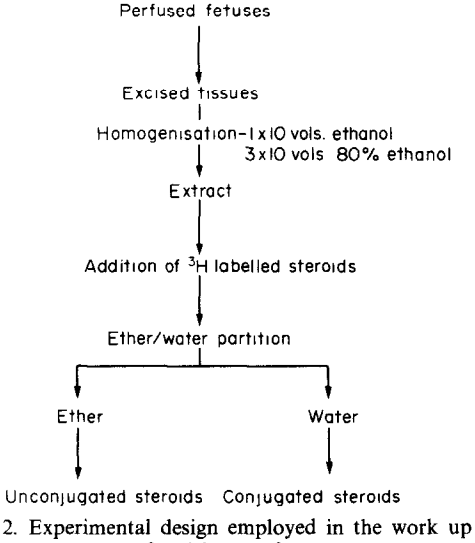

Fig. 2. Experimental design employed in the work up of perfused human fetuses.

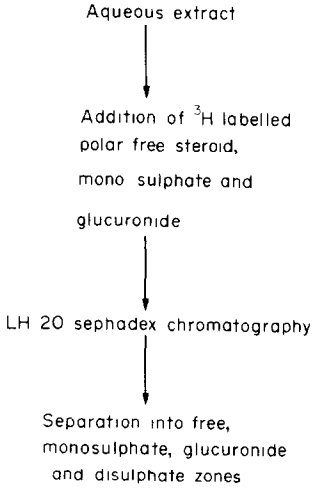

Fig. 4. Fractionation of conjugated steroids.

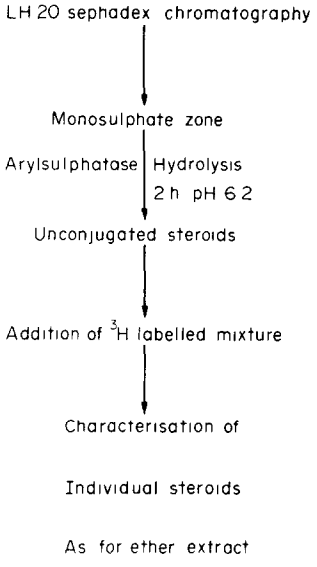

Fig. 5. Fractionation of conjugated steroids (continued).

adrenal extract on LH20. In this instance distinct peaks were obtained for free steroids and monosulphates fractions. The free fractions from all aqueous extracts only contained metabolites which were quantitated from the unconjugated extracts. Apart from these 'free' steroids the only major peak was associated with the monosulphate fraction. The fractions in each monosulphate zone were combined and the monosulphate fractions were subjected to arylsulphatase hydrolysis at pH 6.2 using an enzyme from *Helix pomatia* (Sigma Ltd.). Losses of the monosulphates during LH20 chromatography and the efficiency of the enzyme hydrolysis were computed from recovery data on the internal monosulphate standard added to the aqueous extract prior to chromatography. The mixture of ^{3}H labelled unconjugated steroids was added to the free steroid extracts from the monosulphate zones, after hydrolysis, and the individual steroids present in these extracts were fractionated, characterised and quantitated as described above for the unconjugated extracts.

RESULTS

Table 1 lists the unconjugated corticosteroids isolated from the fetal adrenals in the presence and absence of ACTH. The Table also gives the range of values found in the 2 groups. 11 Deoxycortisol and cortisol were isolated from all perfused fetuses; deoxycorticosterone and corticosterone each from 7 of the 8 fetuses and cortisone from only one fetus in each group. In addition to the compounds listed in Table 1 a number of hydroxylated progesterones including 17α-hydroxyprogesterone were isolated from all fetuses. In the presence of ACTH there was significant increase in the amount of unconjugated corticosterone ($P < 0.05$); increases which were not statistically significantly in unconjugated 11 deoxycortisol and deoxycorticosterone and no significant change in the amount of isolated cortisol which was the major unconjugated corticosteroid isolated. No significant quantities of unconjugated corticosteroids were isolated from any of the perfusates—with or without ACTH.

Since many workers have shown the capacity of human fetal adrenals to conjugate steroid substrates [13] it was of interest to examine the conjugated steroid extracts from these perfusions. Table 2 shows the percentage of the total fetal adrenal activity ± S.E.M., which was in an aqueous soluble form, in the presence and absence of ACTH. This Table also lists the percentage of the total fetal adrenal activity which was isolated from the combined steroid monosulphate zones.

ACTH caused a significant increase ($P < 0.01$) in both the amount of aqueous soluble material and in the amount of activity in the monosulphate zone. The monosulphate zones from the adrenal contained > 70% of the activity associated with the aqueous soluble material. Similar data to that shown for the adrenals in Table 2 is shown for the perfusates in Table 3. ACTH again caused a significant increase ($P < 0.01$) in both the amount of aqueous soluble material in the perfusates and in the percentage activity associated with the perfusate monosulphate

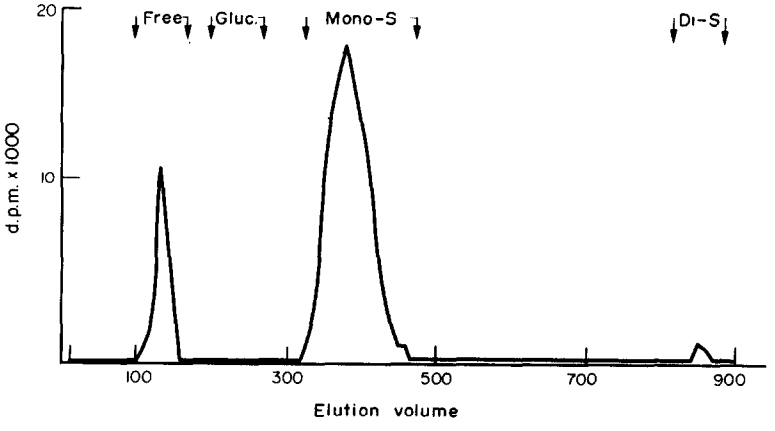

Fig. 6. Elution pattern obtained on chromatography of fetal adrenal aqueous extract on LH20 sephadex.

Table 1. Corticosteroids isolated from unconjugated extracts of fetal adrenals following perfusion of [4^{14}C]-progesterone

	% of total ^{14}C in adrenal			
	−ACTH		+ACTH*	
Metabolite	Mean	Range	Mean	Range
11 Deoxycortisol	2.1	1.0 to 3.3	3.2	1.0 to 7.2
Cortisol	9.2	4.4 to 12.9	8.5	4.9 to 12.2
Cortisone		— to 7.3		— to 12.3
Deoxycorticosterone	1.1	0.3 to 1.9	1.8	— to 2.6
Corticosterone	0.8	— to 1.9	2.0	0.9 to 3.9

* Cortrosyn (1.5 mg/100 ml or perfused blood.
— looked for but not found.

Table 2. Content of aqueous fraction of extracts from fetal adrenals after perfusion with [4-^{14}C]-progesterone

	% of total adrenal ^{14}C aqueous soluble	% of total adrenal ^{14}C extract in monosulphate fraction
ACTH absent	15.7 ± 3.1 (S.E.M.)	12.5 ± 2.2 (S.E.M.)
ACTH present	22.8 ± 2.8 (S.E.M.)	16.7 ± 2.9 (S.E.M.)

zones. In the case of the perfusates only 20–30% of the aqueous activity was in the monosulphate zones. However, this is not surprising since the majority of the activity associated with the perfusate was unconverted substrate. Despite the fact that the monosulphate zones contained only a small percentage of the total activity in the perfusate, these fractions actually contained considerably more ^{14}C radioactivity than was present in the equivalent monosulphate zones from the corresponding fetal adrenals.

Tables 4 and 5 show the corticosteroids isolated from the monosulphate zones of the adrenals and perfusates respectively in the presence and absence of ACTH. In the case of the adrenals only 2 compounds, deoxycorticosterone sulphate and corticosterone sulphate were isolated. From the perfusate monosulphate zones 11 deoxycortisol sulphate was isolated in addition to the above 2 compounds.

ACTH caused a significant increase in the amounts of the sulphates of deoxycorticosterone and corticosterone isolated from the adrenals and a significant increase in the amounts of these 2 compounds and 11 deoxycortisol isolated from the perfusates. In all 5 cases P was <0.001. Although a search was made for other corticosteroid sulphates, including cortisol sulphate, no trace of any of these other steroid sulphates was found in the extracts from the adrenals or perfusates.

Table 6 lists the total mean corticosteroids (unconjugated plus conjugated) isolated from the adrenal extracts in the presence of ACTH. ACTH causes a significant increase in the amount of corticosteroid synthesised ($P = <0.05$).

Since the corticosteroid sulphates isolated from the perfusate were presumably of adrenal origin it is of interest to express the activity associated with these compounds as a percentage of the total adrenal activity.

Table 7 lists the steroid sulphates isolated from the perfusates as a percentage of the total adrenal activity. ACTH causes a 14 fold increase in the total activity isolated as corticosteroid sulphates in the perfusates and the amount in the perfusates is the equivalent, in the presence of ACTH, of 268.32% of the total adrenal activity.

DISCUSSION

The results obtained in this study confirm that the mid-pregnancy human fetal adrenal synthesises corticosteroids from progesterone and sulphoconjugates

Table 3. Content of aqueous fraction of extracts of perfusates after fetal perfusion with [4-^{14}C]-progesterone

	% total perfusate ^{14}C aqueous soluble	% of total perfusate ^{14}C extract in monosulphate fraction
ACTH absent	2.34 ± 0.53 (S.E.M.)	0.43 ± 0.19 (S.E.M.)
ACTH present	11.75 ± 1.81 (S.E.M.)	2.91 ± 0.85 (S.E.M.)

Table 4. Compounds isolated from monosulphate fraction of fetal adrenal extracts after perfusion with [4-^{14}C]-progesterone

	% of total ^{14}C in adrenal	
	−ACTH	+ACTH
Deoxycorticosterone sulphate	4.5 ± 1.5 (S.E.M.)	7.9 ± 0.8 (S.E.M.)
Corticosterone sulphate	3.7 ± 0.9 (S.E.M.)	5.6 ± 1.4 (S.E.M.)

Table 5. Compounds isolated from monosulphate fraction of perfusate extracts after fetal perfusion with [4-^{14}C]-progesterone

	% of total ^{14}C in perfusate	
	−ACTH	+ACTH
Deoxycorticosterone sulphate	0.27 ± 0.12 (S.E.M.)	1.46 ± 0.39 (S.E.M.)
Corticosterone sulphate	0.09 ± 0.03 (S.E.M.)	0.85 ± 0.34 (S.E.M.)
11 Deoxycortisol sulphate	0.03 ± 0.02 (S.E.M.)	0.13 ± 0.05 (S.E.M.)

these corticosteroids. This biosynthesis of corticosteroids is dramatically stimulated by synthetic ACTH. In the presence of ACTH there is a slight increase in the amount of unconjugated corticosteroids isolated. However, the dramatic increase in corticosteroids is observed as monosulphate esters present mainly in the perfusates. The perfusate fractions contain approximately ten times the amount of corticosteroids isolated from the adrenal glands. Since there is no evidence of a major alternative source of corticosteroid synthesis these results show that the fetal adrenal is very active in biosynthesis, sulphoconjugation and secretion of corticosteroid sulphates.

In the adrenals, alone, the effect of ACTH was to increase the formation of corticosterone and deoxycorticosterone and their respective sulphates. In the perfusates, under ACTH stimulation, in addition to the above compounds, increased amounts of 11 deoxycortisol were also found. Although cortisol was the major unconjugated corticosteroid isolated, no stimulation of this compound was found in the presence of ACTH and cortisol sulphate was not isolated from any of the adrenals or perfusates. ACTH therefore stimulates formation of 21 hydroxylated and 21, 11 dihydroxylated compounds with only a relatively small increase in 17α hydroxylated compounds. However, the isolation, in all experiments, of 17α hydroxyprogesterone from the adrenal extracts and the formation of 11 deoxycortisol sulphate suggests that 17α hydroxylation is not a rate limiting step. Perhaps in the mid pregnancy fetus, in contrast to the postnatal human, cortisol is not the most important glucocorticoid. In support of such a hypothesis, it has been suggested that fetal cortisol is largely maternal in origin since the levels of cortisol in cord blood from an anencephalic fetus are similar to those found in

Table 6. Total corticosteroids isolated from fetal adrenals following perfusion with [4-^{14}C]-progesterone

	% of total ^{14}C in adrenal	
	−ACTH	+ACTH
11 Deoxycortisol	2.1	3.2
Cortisol	9.2	8.5
Cortisone	tr	tr
Deoxycorticosterone	1.1	1.8
Deoxycorticosterone sulphate	4.5	7.9
Corticosterone	0.8	2.0
Corticosterone sulphate	3.7	5.6
Total	21.4	29.0

Table 7. Corticosteroid monosulphates isolated from fetal perfusates expressed as % of total adrenal extract

	% of total adrenal extract	
	−ACTH	+ACTH
Deoxycorticosterone sulphate	11.62 ± 4.64 (S.E.M.)	154.33 ± 34.12 (S.E.M.)
Corticosterone sulphate	5.47 ± 2.75 (S.E.M.)	101.26 ± 37.70 (S.E.M.)
11 Deoxycortisol sulphate	1.90 ± 1.04 (S.E.M.)	12.74 ± 5.35 (S.E.M.)
Total of above	18.98 ± 8.11 (S.E.M.)	268.32 ± 63.69 (S.E.M.)

the normal fetus despite the atrophic adrenal cortex of the anencephalic. [14]

Alternatively the fetus may normally make cortisol by a pathway which does not involve progesterone. Since a fetal *de novo* synthesis of pregnenolone does occur [10] a 5-ene-pathway to cortisol might enable the fetus to exert more control over the synthesis of this glucocorticoid.

The results of this study show that ACTH in the fetal circulation is capable of stimulating the fetal adrenal to produce increased amounts of corticosteroids from progesterone. The onset of labour may be regulated by the fetus and the hypothesis is, that fetal pituitary ACTH stimulates the adrenal production of corticosteroids which act on the placenta causing a reduction in progesterone production and an increase in oestrogen production. These changes are then related to secretion of prostaglandins and oxytocin which finally initiate myometrial activity. As a result of this study the feasibility in the human of the fetal part of such a pathway is confirmed.

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DISCUSSION

Swaab. I should like to say that the human fetal pituitary is not involved in the same way in the initiation of parturition as in sheep. We have investigated about 150 anencephalic fetuses and found that the mean pregnancy length in anencephaly without hydramnios is not prolonged. So in this respect the human differs from the fetal sheep model. In addition, the distribution of the moment of birth of anencephalic fetuses showed a difference from that of the normal group, such, that about 30% delivered premature and 30% postmature (Honnebier and Swaab, 1973). The same phenomenon has been found by Novy (1977) in rhesus monkeys from which the brain and the pituitary were removed. So it seems as if the fetal brain in men and monkey might have a role in the exact timing of the moment of labour but certainly in a different way than in sheep.

Macnaughton. This was human, other people have found it was prolonged I think. Were you able to measure corticosteroids in the umbilical blood of anencephalics?

Swaab. The corticosteroid levels we measured in anencephalic cord blood were similar to those in normal children. So the corticosteroid level in cord blood is probably not a reliable index of fetal adrenal function.

Macnaughton. This difference of information about the anencephalic is quite puzzling because many workers have reported that pregnancy is prolonged and some say it's not. We really are pretty much in the dark about this.

Swaab. In all the anencephalics in our study the cranial vault was completely or almost completely missing while the "cerebral tissue" was composed of small irregular masses or vascular structures only. The entire group of anencephalics had a mean pregnancy length of 36.6 weeks. This figure is of course influenced by many factors e.g. hydramnios, fetal death, twin-pregnancy and by induction of labour. The group of anencephalics in which all such cases were omitted and that delivered spontaneously had a normal mean pregnancy length (39.7 weeks). This is against the general opinion that in the absence of hydramnios anencephaly would be associated with prolonged pregnancy (for details see Honnebier and Swaab, 1973).

Pasqualini. Concerning the significant quantity of deoxycorticosterone sulfate and corticosterone sulfate you found in the fetus. I remember that in studies with the biosynthesis of these two steroid ester-sulfates we found differences in the sulfokinase activity for deoxycorticosterone and corticosterone in the fetal compartment (*Excerpta Medica. Int. Congr* Ser. **219** (1971) 487–495). Do you have some further news about this problem? Secondly, do you have some data on how much of deoxycorticosterone sulfate or corticosterone sulfate is circulating in the bound or unbound forms?

Macnaughton. I have no further information about the first, and we haven't in fact looked at the binding of these.