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SHORT COMMUNICATION

CONVERSION OF CORTICOSTERONE 21-SULPHATE INTO 5α-TETRAHYDROCORTICOSTERONE SULPHATE BY FOETAL RAT LIVER*

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SUMMARY

After *in vivo* perfusion of rat foetuses with [^{3}H]corticosterone 21-sulphate, 40–45% of the radioactive material in the foetal liver consisted of 3α,11β,21-trihydroxy-5α-pregnan-20-one sulphate.

IN A PREVIOUS paper it was demonstrated that after administration of [^{3}H] corticosterone to rats, a significant part of this hormone and its metabolites is present in the liver and intestinal tissues as sulphate esters[1]. Recently, the conversion of corticosterone to corticosterone sulphate was shown to occur in rat foetal liver[2].

In the present investigation, 0·9 μCi of sodium [1,2-^{3}H]corticosterone 21-sulphate (11β-hydroxy-4-pregnene-3,20-dion-21-yl-sulphate, sodium salt), S.A. 12·4 mCi/mmol, was prepared as previously described[3], dissolved in 0·1 ml NaCl solution (9:1000, w/v) and injected subcutaneously *in vivo* and *in situ* to each of 13 foetuses of two Wistar rats at 17–19 days of gestation. After 30 min, the foetal (liver, intestines, lungs, spleen, kidneys and brain) and placental tissues were separated and extracted successively with 80% and 90% ethanol. The ethanol extracts were dried, dissolved in water, the unconjugated material extracted with dichloromethane and the conjugates with n-butanol. After control by: (1) paper chromatography in the system butyl acetate: n-butanol: toluene: 4N ammonium hydroxide: methanol (6:1:3:5:5, by vol.), (2) paper electrophoresis, (3) solvolysis and (4) sulphatase hydrolysis, it was observed that sulphate esters were the only radioactive components of the n-butanol extracts.

Table 1 shows the recoveries of radioactivity from the different foetal tissues and from the placenta, as well as the distribution of the radioactive material between the free and conjugated fractions. The largest part (9·1%) of the injected dose was localized in the foetal liver; in all the tissues the greatest part of the radioactive material (92–100%) was found in the sulphate fraction.

A part of the sulphate fraction of the foetal liver tissue was solvolyzed[4] and the free material was chromatographed in the system chloroform/formamide. The area corresponding in mobility to 5α-tetrahydrocorticosterone (3α,11β,21-trihydroxy-5α-pregnan-20-one) was eluted and chromatographed successively in

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Table 1. Distribution of the radioactive material in the different foetal tissues and in the placenta, and between the free and sulphate fractions, after [^{3}H]corticosterone-21-sulphate administration to rat foetuses

Tissues	Recovery of the administered dose (%)	Radioactive material in each tissue	
		Free (%)*	Sulphate (%)*
Foetus			
Liver	9·10	1·5	98·5
Lung	0·80	4·0	96·0
Spleen	0·08	0	100
Kidney	0·90	0	100
Brain	0·60	8	92·0
Placenta	2·30	1·3	98·7

*Values are expressed as percentage of the total radioactivity in each tissue.

Table 2. Successive recrystallizations with authentic 5α-tetrahydrocorticosterone of the corticosterone metabolite isolated in the sulphate fraction of the rat foetal liver

Solvents	Crystals (d.p.m./mg)	Mother liquors (d.p.m./mg)
Methanol	2100	3160
Methanol/ethanol	2040	2150
Methanol/benzene	2060	2080

the systems: toluene/propanediol, benzene/formamide and isooctane: toluene: methanol: water (2:8:5:5, by vol.). In each of these systems the radioactive material had the same R_F value as authentic 5α-tetrahydrocorticosterone. An aliquot of this material was acetylated; the resultant acetate had the same polarity as 3α,11β,21-trihydroxy-5α-pregnan-20-one 3,21-diacetate in the chromatographic systems ligroin/propanediol and isooctane: toluene: methanol: water (7:1:4:4, by vol.). Another aliquot of the freed radioactive material from the same sulphate fraction was mixed with 20 mg of authentic 5α-tetrahydrocorticosterone and crystallized. The results of the crystallization are shown in Table 2. Quantitative evaluation established that 40–45% of the radioactive material present in the foetal rat liver tissue consisted of 5α-tetrahydrocorticosterone sulphate.

Since in the other foetal tissues, as well as in the foetal liver, very little radioactive material was found in the free form (Table 1), it is suggested that the 5α-tetrahydrocorticosterone found in the sulphate fraction is a direct metabolic conversion product of the administered corticosterone sulphate. It is interesting that in man, corticosterone sulphate can be converted, without previous hydrolysis, into sulphates of different corticosterone metabolites [3] and that a 'direct metabolism' of corticosterone sulphate to the sulphates of 3α,11β,21-trihydroxy-5β-pregnan-20-one and 3β,11β,21-trihydroxy-5α-pregnan-20-one was demonstrated in *in vitro* studies with adult rat liver [5].

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