Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 859–864. Pergamon Press. Printed in Great Britain

STEROID DEHYDROGENASES IN RABBIT LIVER OF VARIOUS DEVELOPMENTAL STAGES

A. M. BONGIOVANNI and J. MARINO
Children's Hospital of Philadelphia, Department of Pediatrics, University of Pennsylvania, 1740 Bainbridge Street, Philadelphia, Pennsylvania 19146, U.S.A.

(*Received* 13 *May* 1972)

SUMMARY

The enzymatic systems generally termed 3β, 3α- and 17β-hydroxy-steroid oxidoreductase have been examined in the liver of rabbits of various ages. Livers of the rabbit fetus of 30–33 days gestation is virtually inactive in all of these transactions. The relative inefficiency of these enzymes may in part explain the peculiar steroidal patterns of mammalian fetal and neonatal life.

INTRODUCTION

IN THE course of examining steroidal 3β- and 17β-oxidoreductase activity in rabbit liver certain age differences were noted. Investigations were limited to the employment of C-19 steroidal substrates, 5α-androstane-3,17-dione and 3β-hydroxy-5α-androstan-17-one. It is the purpose of this report to describe the age differences.

EXPERIMENTAL

The livers of male rabbits were removed immediately following death by decapitation and frozen immediately. The fetal tissues were of 30–33 days gestation, the "young" animals of 20 days from birth and the "mature", 17–19 months. The procedure was essentially that of Ungar *et al.*[1], except that in most experiments a whole homogenate was employed rather than the soluble fraction alone after high speed centrifugation. This, by design, in order to detect age differences within more complete tissue homogenates rather than sub-fractions.

The tissues were homogenized in 0·1 M phosphate buffer, pH 7·4, with a Vir-Tis homogenizer, employing 5 ml. buffer per g liver. These were spun at 800 *g* for 30 min and the supernatant employed. In a few initial trials described below the supernatant after centrifugation in a Spinco centrifuge at 59,000 *g* was used and since little difference was noted the remaining trials employed the supernatant after 800 *g*. In a single early trial liver of male adult rats was used and appeared to be less satisfactory than the rabbit.

The incubations entailed volumes of tissue homogenates of 4·0 ml containing 500 ± 20 mg. protein[2] and 750–860 nmol of steroidal substrate. The steroid was added to the extract in 0·05 ml methanol and 400 μg NADH was added. The procedure was carried out in 50 ml Erlenmeyer flasks, at 37°C. In an atmosphere of nitrogen for three hours. All experiments were performed in duplicate.

At the conclusion of the incubation 25·0 ml of acetone were added to each flask and left at 4°C overnight. At this point 250 nmol of 21-hydroxy-5-pregnene-3,20-dione acetate was added as an internal standard for recovery. After filtration through Whatman #4 filter paper, the volume was reduced *in vacuo* to 5·0 and

25·0 ml water was added. This was extracted twice with 50 ml methylene chloride, the solvent was washed two times each with 10 ml of 0·1 N sodium hydroxide and water. The methylene chloride was evaporated to dryness.

Identification of products and quantitation was by gas–liquid chromatography. Preliminary treatment consisted of application of the extract to a column of alumina, water content 4%, 1 × 10 cm in benzene. The column was washed with 10 ml benzene, 15 ml of 1% ethanol in benzene and elution of products for gas chromatography with 4% ethanol in benzene. It was previously demonstrated that the steroidal substrate and all anticipated products would be recovered (95%) in this fraction.

Gas liquid chromatography was carried out in a Barber–Coleman gas chromatograph, model 5000 with a hydrogen flame ionization detector. Suitable aliquots of the final eluates were applied on OV-1 at 232°C. and 22 psi nitrogen, both as the free and acetylated compound. It was required that each product conform to authentic standards both as the free and acetylated derivatives. The quantitation was by planimetry and comparison with standard companion steroids. The results reported were corrected according to the recovery of the internal standard. Actual recoveries however were good, 80–95%.

The steroidal substrates employed in the incubations as well as standards for recognition of products were purchased from Steraloids and Ikapharm. Experiments described herein employed 5α-androstane-3,17-dione and 3β-hydroxy-5α-androstan-17-one as substrates. The results are reported as quantities measured as the acetate derivatives since the individual peaks were better separated than the free. The relative retention times on gas–liquid chromatography with respect to etiocholanolone are shown in Table 1. Nonetheless correspondence was required for both the free and acetylated forms with respect to retention times and the results as reported.

Table 1

	Free	Acetate
5α-androstane-3,17-dione	1·189	1·189
5α-androstane-3α-ol	1·098	1·378
5α-androstane-3β-ol	1·100	1·555
5α-androstane-3α,17β-diol	1·108	1·955
5α-androstane-3β,17β-diol	1·133	2·222

Gas chromatographic characteristics of steroids employed in this study. Results shown as relative retention times (RT) with reference to etiocholanolone on OV-1.

RESULTS

In preliminary experiments shown in Table 2 and Fig. 1, it can be seen that rabbit liver was more active with respect to 3α- and 3β-reduction as well as 17β-reduction. At no time was 17α-reduction noted. These results also demonstrate little difference between the soluble fraction after centrifugation at 59,000 *g* and the more "complex" liver extract. Thus the decision was made to employ the cruder preparation to determine age differences.

Table 3 and Fig. 2 compare the results of activity between fetal, young and mature rabbits when androstanedione was the substrate. The fetal liver is con-

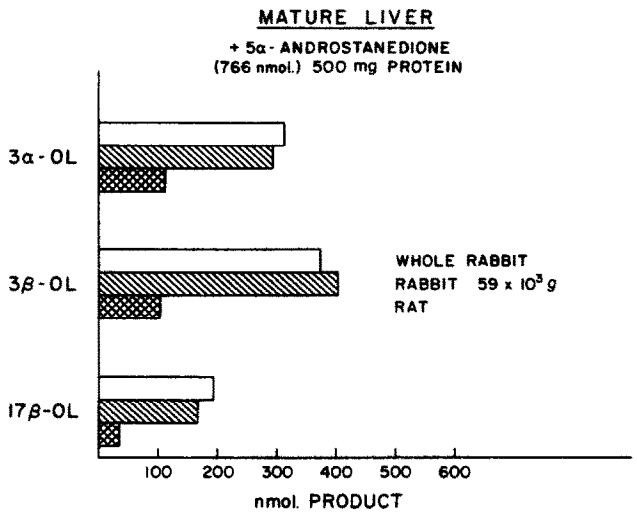

Fig. 1. Illustrates total nmoles of substrate reduced at 3β and 17β positions by rabbit liver supernatant after 800 *g* centrifugation (whole rabbit) 0, supernatant of rabbit after 59,000 *g* and rat after 800 *g*. All animals males and sexually mature. See Table 2 for details.

Table 2. Duplicate incubations with 5α-androstane-3,17-dione as substrate

Tissue	Rabbit 59,000 *g* Supernatant		Rabbit 800 *g* Supernatant		Rat
		Control		Control	
Substrate*	766	0	766	0	766
Products					
A-dione†	96–111	0	5–10	0	100–200
3α-ol	260–276	0–11	250–300	0–10	75–82
3β-ol	89–96	0	124–140	0	89–94
3α,17β-diol	26–35	0	55–64	0	24–31
3β,17β-diol	72–86	0	122–139	0	70–90

All results shown in nmols. All tissue from mature animals.

*Indicates amount of substrate added per 500 mg. protein. Also shown are trials without any substrate. Abbreviations used in all tables: A-dione = 5α-androstane-3,17-dione; 3α-ol = 3α-hydroxy-5α-androstan-17-one; 3β-ol = 3β-hydroxy-5α-androstan-17-one; 3α,17β-diol = 5α-androstane 3α, 17β-diol; 3β,17β-diol = 5α-androstane-3β,17β-diol.

†Unchanged substrates.

siderably less active in all reductions and particularly so with regard to 17β-dehydrogenase. In the tables and figures, results are shown for duplicate experiments. There is little difference between young and mature animals. It is apparent that some small amount of 3α- and 3β-oxireductase performance is to be found in the fetus. The "young" liver is perhaps better able to reduce the molecule to 17β-hydroxy than the more mature. The experiments are too few however to attach significance to this difference. Table 2 also shows results under the experimental conditions with no substrate added.

Table 4 and Fig. 3 demonstrate the behavior among the age groups when a 3β-

Table 3. Incubations of 5α-androstane-3,17-dione with rabbit liver of various ages

Rabbit tissue	Fetal	Young	Mature
Substrate added	750	750	750
Products			
A-dione	510–562	0	0
3α-ol	57–79	81–89	140–216
3β-ol	119–197	163–175	292–311
3α,17β-diol	0	46–57	50–68
3β,17β-diol	0	220–232	118–158

All results shown in nmol. Duplicate results shown.

The sum of all 3α reduction is the total of 3α-ol + 3α,17β-diol. So too with 3β-reduction. This is reflected in Fig. 2.

Table 4. Incubations of 3β-hydroxy-5α-androstan-17-one with rabbit livers of various ages

Rabbit tissue	Fetal	Young	Mature
Substrate added	860	860	860
Products			
Substrate	700–716	334–355	180–195
3α-ol	0–34	69–80	39–80
3α,17β-diol	3–11	31–40	170–179
3β,17β-diol	14–25	385–395	365–382

All results in nmols. Duplicate results shown.

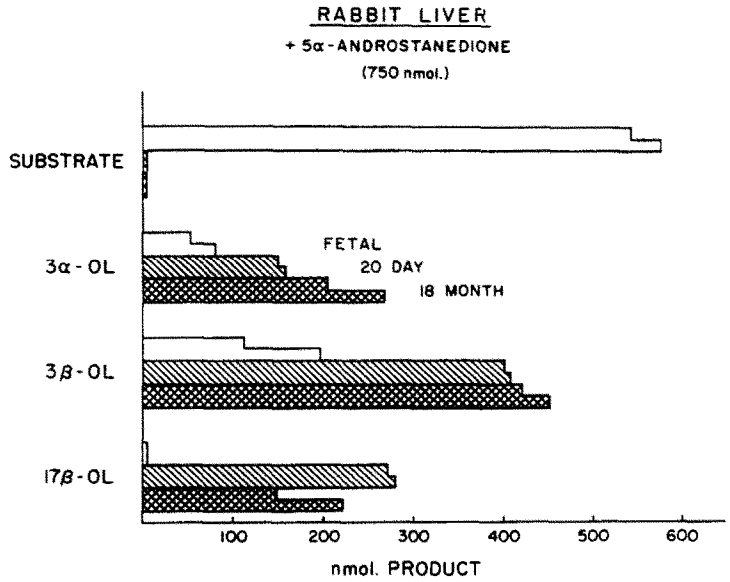

Fig. 2. Shows total steroidal products as nmols. reduced at 3α- and 17β-positions by fetal, post-natal and mature rabbit livers with 5α-androstane-3,17-dione as substrate. See Table 3 for details.

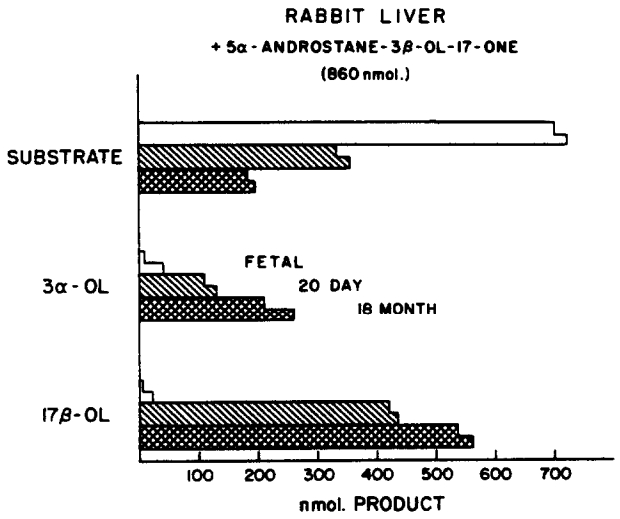

Fig. 3. Shows total steroidal products as nmols. products with rearrangement of 3β-ol to 3α-ol and 17β-reduction with 3β-hydroxy-5α-androstane-17-one as substrate. See Table 4 for details.

hydroxy substrate is employed. The fetal liver demonstrates virtually no activity whatsoever. The mature animals are perhaps better able to rearrange the 3β-hydroxyl function from 3β to 3α but again the difference may not be significant. As with the previous experiments it is the fetal liver which is clearly different. In this set of experiments no other products whatsoever were detected by gas chromatography so that none of the possible intermediates between 3β-hydroxy and 3α-hydroxy steroids were perceived. This may be related to the length of incubation and perhaps the total reaction is rapid. At no time were 17α-hydroxy products noted although studies with such standards would have enabled their recognition.

In several experiments the substrate 3β, 17α-dihydroxy-5-pregnen-20-one was employed in the hope of discovering its conversion to 5β-pregnane-3α,17α,20α-triol. However the substrate remained unchanged and was recovered in all age groups in its original form.

DISCUSSION

It is apparent from these experiments that the fetal rabbit liver is relatively inactive with respect to 3α, 3β and 17β oxidoreductase activity. Indeed the fetal liver is able to demonstrate some little 3β-reductase activity on a 3-keto substrate while it shows no ability to handle to steroid with a 3β-hydroxyl function. Probably there are several different enzymes involved, all too freely termed 3β-oxidoreductase. This matter requires further clarification. In all studies the fetal liver seems to have no 17β-oxidoreductase activity.

It is now well recognized that 5-ene-3β-hydroxy steroids predominate in the mammalian fetus. There are several propositions set forth to explain this. The fetal adrenal secretes these steroids as sulfates and the fetus is lacking in sulfatases so that the appropriate "free" compound is unavailable for transmutation. It would also appear to be true that necessary steroidal dehydrogenases are also lacking and appear sometime after birth. It is especially notable that a 17β-oxidoreductase

is absent from fetal liver, the significance of which is not clarified from these studies.

One of the instigations for this exploration concerns the human disease of adrenal hyperplasia due to a deficiency of 3β-hydroxy steroid oxidoreductase [3]. Although in this disorder 5-ene-3β-hydroxy steroids predominate, it has been noted that in older subjects who survive infancy, the urine may contain respectable quantities of 5β-pregnane-3α,17α,20α-triol [4, 5]. These results suggest that as the subject with this disorder matures his liver may be able to transform 3β-hydroxy steroids and that the hepatic enzyme is under different genetic control than the enzyme system in steroidogenic organs.

In conclusion it should be stated that these experiments were simplified by the notable lack of numerous unidentifiable compounds upon gas–liquid chromatography. There were few substances detected, all conforming to expected products. One exception is noted in passing. When androstanedione was the substrate, very small quantities of 11-hydroxylated derivatives were noted in the extracts of young and mature, but not fetal liver. These results are not detailed because the amounts were small and the identity of these products was not rigidly confirmed. Otherwise there were no peculiar products to complicate the analysis of the results.

ACKNOWLEDGEMENTS

This study was supported by Research Grant HD-00371, Training Grant AM-05197 of The National Institutes of Health, U.S. Public Health Service.

The authors are indebted to Mr. Leon Cienawa for technical assistance.

REFERENCES

1. Ungar F., Goldstein M. and Kao C.: *Steroids, Suppl.* 1, (1965) 131.
2. Lowry O. H.: *J. Lab. clin. Med.* 39 (1952) 663.
3. Bongiovanni A. M.: *J. clin. Invest.* 41 (1962) 2086.
4. Zachmann M., Völlmin J. A., Murset G., Curtius H. and Prader A.: *J. clin. Endocr.* 30 (1970) 719.
5. Jänne O., Perheentupa J. and Vihko R.: *J. clin. Endocr.* 31 (1970) 162.