Steroid Δ^4-Hydrogenases of Rat Liver.* (24430)

E. FORCHIELLI, K. BROWN-GRANT† AND R. I. DORFMAN
Worcester Foundation for Exper. Biology, Shrewsbury, Mass.

Previous studies with fractions prepared from livers of adult male rats have demonstrated presence of both a soluble Δ^4-5β-hydrogenase and a microsomal Δ^4-5α-hydrogenase(1). Development of a quantitative method for assay of these enzymes(2,3) has made possible a study of the effective enzyme concentrations in liver with respect to such factors as sex, age and endocrine status.

Methods. Animals were sacrificed by stunning and decapitation and the livers rapidly removed and chilled on ice. All subsequent operations were carried out at 2-3°C. Tissues were homogenized by a VirTis blendor at full speed for 20 seconds with one or 1.5 volumes of either 0.25 M sucrose or 0.154 M KCl as homogenizing medium. The various tissue fractions were prepared by differential centrifugation in a Spinco Model L centrifuge. The usual incubation procedure was as follows: a volume of each tissue preparation equivalent to 100 mg wet weight of whole liver plus 0.5 μM of steroid substrate was incubated with 1 μM TPN, 5 μM glucose-6-PO_4, 0.5 K unit glucose-6-PO_4 dehydrogenase, 10 μM $MgCl_2$, 20 μM phosphate buffer pH 7.2, and 0.154 M KCl to make a final volume of 1 ml; the incubation periods were for 10 minutes (unless otherwise indicated) at 37° in an atmosphere of nitrogen. The reaction was stopped by rapid chilling of the incubation tubes and steroid substances were extracted with 4 ml redistilled methylene dichloride. Recoveries in control experiments averaged 95%. This value was used in calculation of reaction rates which were determined by measuring the loss of the characteristic absorption near 240 mμ. All determinations were performed in triplicate. Nitrogen determinations were made in duplicate by a micro-Kjeldahl method.

Results. Comparison of reducing activity of male and female rat livers. A marked quantitative difference was observed in steroid Δ^4 reducing activity of whole homogenates prepared from livers of adult male and female rats when 11-deoxycortisol was used as a substrate. The activity of female livers was consistently higher whether expressed as μM of substrate reduced /100 mg wet weight equivalent or /mg nitrogen (Table I). In addition to the quantitative difference, a striking qualitative difference was observed. In the female, Δ^4 reducing activity was found only in the microsomal fraction. No evidence for the 5β soluble enzyme could be obtained in female liver preparations but this enzyme is easily demonstrable in male rat liver(1,4). The difference in level of Δ^4 reducing activity observed in whole homogenates of male and female livers was also seen when the activity of the microsomal fractions was compared (Table I). Ratio of activity (female/male) was only slightly lower than that seen with whole homogenates.

The microsomal 5α-enzyme prepared from the female liver appeared to be the same as that separated from the male liver with respect to pH optimum (7.0-7.5) stability and similar relative rates of reduction with various steroid substrates (Table II). It may be noted that the ratio of Δ^4 reducing activity between male and female was quite constant, whether the activities were compared in whole homogenates (not shown) or in microsomal fractions. This suggests that under the conditions of these experiments Δ^4 reducing activity of male rat liver is predominantly due to the 5α microsomal enzyme. Indeed, when the soluble enzyme was prepared free from particulate material by centrifugation at 105,000 x g, only 18-21% of the whole homogenate activity was found in this fraction. In confirmation of the findings of Tomkins(4), the soluble 5β enzyme was found to exhibit an acid pH optimum (5.5-6.0) and in the un-

* These investigations were supported in part by grants from U. S. Atomic Energy Comm.; U.S.P.H.S.; and Nat. Cancer Inst., U.S.P.H.S.

† Present address: Department of Physiology, Univ. of Birmingham, England. Dr. Brown-Grant gratefully acknowledges assistance of Wellcome Foundation of Travel Grant.

TABLE I. Comparison of Reducing Activity of Whole Homogenates and Microsomal Fractions of Male and Female Rat Livers. Substrate: 11-Deoxycortisol.

Type of preparation	No. of pairs	$\mu M \times 10^3$ steroid reduced/100 mg wet wt equivalent (mean ± S.E.) ♂	♀	Ratio*	$\mu M \times 10^3$ steroid reduced/mg nitrogen (mean ± S.E.) ♂	♀	Ratio*
Whole homogenate	6	94 ± 8.3	349 ± 30.0	3.8 ± .44	76 ± 6.5	274 ± 27	3.7 ± .31
Microsomal	6	53 ± 5.4	188 ± 33.3	3.5 ± .44	147 ±14.7	536 ±137	3.4 ± .60

* Ratio = female/male.

TABLE II. Relative Rates of Δ^4-Reduction (μM per Min.) of Various Natural and Synthetic Steroids by Microsomal 5α Enzyme Prepared from Male and Female Rat Livers and by Soluble 5β Enzyme from Male Rat Liver. (Results expressed in terms of cortisol = 100. Incubations were for 30 min. with 80 μg steroid. Actual reductions of cortisol were 0.105, 0.112 and 0.097 μM in 30 min. for female 5α, male 5α and male 5β enzyme respectively.)

Substrate	Type and source of enzyme preparation ♀ microsomal	♂ Microsomal	♂ Soluble
Cortisol	100	100	100
11-Deoxycortisol	91	80	67
Deoxycorticosterone	154	141	34
Progesterone	126	130	35
Δ^1-Dehydrocortisol	14	4	13
2α-Methylcortisol	0	0	0
Δ^4-Androstene-3,17-dione	179	171	146
Testosterone	164	155	83
19-Nortestosterone	87	101	64
17α-Methyl-19-nortestosterone	108	105	48

purified state to be active with a variety of steroid substrates. The relative rates, however, were quite different from those observed in the case of the 5α microsomal enzyme (Table II).

Effect of ovariectomy and hypophysectomy in female rats on levels of microsomal 5α-enzyme. The sex differences suggested the possibility that the gonads may be involved in control of Δ^4 reducing enzyme activity in rat liver. Accordingly, female rats were ovariectomized at 21 days of age and when examined 28 and 33 days later showed only a slight but consistent drop of 10% in titer of the 5α microsomal enzyme. Animals injected subcutaneously with a daily dose of 50 μg of estradiol-17β benzoate showed no change from control values. Hypophysectomized female rats (140-150 g initial body weight) showed a 73% drop in reducing activity 10 days after operation. Thirty days after operation no reducing activity was detectable in the microsomal fraction. Loss in activity was reduced about 50% by administration of ACTH (1.0 I.U./day), pregnant mare serum (10 I.U./day) or growth hormone (Horner, 10 μg/day) for 10 days.

Variations in microsomal 5α-enzyme with age in female rats. Striking differences were noted in the 5α reducing activity in livers from female rats of different age groups ranging from 10 days to more than 18 months (Table III). The low enzyme titer in the 10-day-old rats was increased threefold in 15 to 21-day-old animals. Between the 21st and 43rd days of life the enzyme content was again tripled. This high level of activity remained constant at least until the rats were 11 months old. A significant drop, however, was observed in a group of animals older than 18 months.

Discussion. There is no explanation at present for the marked qualitative and quantitative differences in concentration of liver Δ^4-hydrogenases between female and male rats. The possibility of a gonadal control of these enzymes, while not excluded, received little support from ovariectomy experiments in the female. Furthermore, a sharp increase in enzyme titer takes place before sexual maturation in the female, which again argues

TABLE III. Variations of 5α Microsomal Enzyme Activity in Livers of Female Rats of Different Ages (One Hr Incubation).

Age (days)	No. of rats	11-Deoxycortisol reduced (%)
10	20	8
15	18	24
21	15	26
27	8	67
43	2	85
97	3	88
330	6	88
>540	3	25

against a major role for the ovary in control of the 5α enzyme level. The more marked effects of hypophysectomy are difficult to interpret, especially as several trophic hormones were able in some degree to restore the levels of activity toward normal.

Summary. 1. The rate of Δ^4 reduction of 11-deoxycortisol was 3-4 fold greater in female than in male rat liver whole homogenates. The same sex difference was found in microsomal fractions containing the Δ^4-5α-hydrogenase. 2. Evidence was found for only one Δ^4-hydrogenase (5α-microsomal) in female rat liver in contrast to the male liver which contains the soluble Δ^4-5β-hydrogenase as well. 3. Ovariectomy of adult female rats did not produce any marked change in enzyme titer. Hypophysectomy resulted in a sharp drop which could be reversed in part by ACTH, growth hormone or pregnant mare's serum. 4. Rapid increases in the titer of Δ^4-5α-hydrogenase in the liver of the female rat occurred prior to the known stages of sexual maturation.

1. Forchielli, E., Dorfman, R. I., *J. Biol. Chem.*, 1956, v223, 443.
2. ———, Endocrine Soc., 39th Meeting, N. Y., 1957.
3. ———, *Fed. Proc.*, 1958, v17, 222.
4. Tomkins, G. M., *J. Biol. Chem.*, 1957, v225, 13.

Received August 6, 1958, P.S.E.B.M., 1958, v99.

Reversal of Hepatic Venous Circulation in Dogs.* (24431)

CHARLES G. CHILD, III; EUGENE F. MCDONOUGH, JR. AND GERARD C. DESROCHERS
(Introduced by J. M. Hayman, Jr.)
Dept. of Surgery, Tufts University School of Medicine and Surgical Service, Boston City Hospital, Boston, Mass.

Much has been learned over the years about the function of liver in animals by surgically altering its normal vasculature. Ligation of the hepatic artery(1), Eck fistula(2), transposition of portal vein and vena cava(3), and partial occlusion of the hepatic outflow tracts (4) have all contributed to a better understanding of the complexities of hepatic function. Several years ago one of us (CGC) conceived that additional information about function and hemodynamics of the liver might be gained were it possible to study for prolonged periods animals whose hepatic venous circulation had been reversed. This report describes experiments in dogs as a result of which all caval blood caudad to the diaphragm enters the liver through the hepatic veins and leaves, together with the hepatic arterial blood, by way of the portal vein. Dogs with this type of reversal of hepatic blood flow have now survived up to 57 days in apparent good health. Didier(5) demonstrated in dogs that the flow of portal blood alone could be reversed for a few hours. He did his experiments at the suggestion of M. Hallion, but did not, as far as we have been able to determine, secure prolonged survival among his animals. Didier's experiments were not known to us when we started our investigations of reversal of hepatic venous circulation.

Materials and methods. Healthy mongrel dogs weighing 12 to 44 kg were anesthetized with intravenous pentobarbital sodium, 60 mg/2.2 kg of body weight. Animals were maintained during open thoracotomy by intermittent positive pressure endotracheal insufflation. A crimped teflon† vascular prosthesis, 1 cm internal diameter and varying in length with size of dog from 11 to 15 cm, was used to maintain free flow of blood from the side of the superior mesenteric vein to the side of thoracic inferior vena cava. After completion of end to side anastomoses, 50 mg of heparin in 500 ml of isotonic saline solution was administered slowly over 3 hours into a convenient vein in the jejunal mesentery. When the vascular prosthesis between superior mes-

* This investigation was supported by research grant from Nat. Heart Inst., U.S.P.H.S.

† C. R. Bard, Summit, N. J.