Influence of Sex and Sex Steroids on Bilirubin Uridine Diphosphate-Glucuronosyltransferase Activity of Rat Liver

MAURIZIO MURACA and JOHAN FEVERY
Laboratory of Hepatology, Department of Medical Research, University of Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium

Bilirubin uridine diphosphate-glucuronosyltransferase activity was markedly higher in liver preparations of adult female rats when compared with adult male rats. Ovariectomy decreased the levels in females whereas orchiectomy produced an increase in males. Totally opposite results were obtained when assaying p-*nitrophenol uridine diphosphate-glucuronosyltransferase; this suggests the existence of different enzymes. Administration of physiologic amounts of testosterone decreased enzyme activities in orchiectomized males to normal levels. Estradiol had no effect but progesterone significantly enhanced the transferase activity of gonadectomized females. The effect was more pronounced when progesterone was associated with estradiol. The combined treatment of ovariectomized rats resulted in physiologic plasma levels of sex steroids and could increase the levels of glucuronosyltransferase to values of normal females. Male and female sex hormones thus exert opposite effects on bilirubin uridine diphosphate-glucuronosyltransferase. If applicable to humans, this might explain the differences in serum bilirubin levels in adult men and women, and in the prevalence of Gilbert's syndrome.*

Serum bilirubin levels are higher in adult men than in women (1,2); however, before the age of 10 such a difference does not exist (1). This suggests that hormonal changes at puberty could interfere with the normal bilirubin metabolism. The key step in bilirubin metabolism is conjugation, catalyzed by a microsomal uridine diphosphate-glucuronosyltransferase (UPD-glucuronosyltransferase). This enzyme, present in various organs but most abundant in the liver (3,4), might well be influenced by sex steroid hormones. Several studies have investigated the effect of sex hormones on the conjugation of exogenous substances (5–13) but little information is available regarding the glucuronidation of the endogenous substrate bilirubin. In the present work, we studied the glucuronoconjugation of bilirubin in the liver of male and female rats of different ages in order to evaluate the development of a sex difference. The influence of sex steroids on the enzyme during adult life has also been evaluated by studying the effects of gonadectomy and of replacement treatment with sex hormones.

Material and Methods

Chemicals

Bilirubin and *p*-nitrophenol were obtained from Merck A.G. (Darmstadt, West Germany) and UDP-glucuronic acid (ammonium salt) from Sigma Chemicals Co. (St. Louis, Mo.).

Animals, Gonadectomies, and Hormonal Treatments

Male and female Wistar R/A rats (inbred strain) were kept at 20°–25°C, with humidity ranging from 45% to 80% and with 10 h of light and 14 h of darkness per day. Animals had free access to food and water, and they were not starved before bilirubin conjugation was studied.

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Address requests for reprints to: Johan Fevery, M.D., Ph.D., Laboratory of Hepatology, Research Building, Campus Gasthuisberg, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium.

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Abbreviations used in this paper: UDP-glucuronosyltransferase, uridine diphosphate-glucuronosyltransferase.

To study the effect of gonadectomy and replacement therapy, postpubertal rats (7-wk-old) underwent orchiectomies or ovariectomies under pentobarbital anesthesia (50 mg/kg body wt; Nembutal, Abbott Pharmaceuticals, Belgium) according to the techniques described by Waynforth (14). Controls were sham-operated. Some gonadectomized males were given testosterone subcutaneously as a long-acting preparation. Testoviron-dépôt (25 mg in 0.1 ml of sesame oil) [testosterone proprionate and oenanthate, Schering, Kamilworth, N.J.] was administered on the day of operation and after 10 days. A similar dose had been found to restore accessory sex tissue weight in castrated male rats (15). Some gonadectomized female rats received the following steroid preparations alone or in combination: (a) 17-β-estradiol (Sigma), 1 mg suspended in 10 μl of sesame oil and sealed in a 1-cm-long medical grade tubing (Silastic No. 602-285, Dow Corning, Midland, Mich.) by a silicon-type medical adhesive (Dow Corning) and implanted subcutaneously in the cervical region on the day of gonadectomy. By this method, serum levels of estrogens in ovariectomized rats were found to be maintained within the physiologic range for at least 6 wk. (b) Progesterone extemporally dissolved in olive oil/ethanol (90:10 by volume) was administered subcutaneously at the empirical dose of 2.5 mg in 0.1 ml twice every day. (c) Searching for a more practical way of administration, progesterone was also given as a long-acting preparation (hydroxyprogesterone caproate, Proluton-dépôt, Schering). Three different dosages, 12.5, 25, and 50 mg, were arbitrarily chosen and were injected subcutaneously on the day of operation. This was followed by a second dose after 10 days. Control animals received vehicle only or were given an implant containing vehicle only. All animals were killed after 21 days of treatment. Serum concentrations of testosterone, 17-β-estradiol, and progesterone were determined by radioimmunoassay (16,17).

Enzyme Assay

Body weights were recorded before the animals were killed by cervical dislocation. Livers were excised and weighed. Homogenates (100 mg of wet liver per milliliter of suspension) were prepared in 250 mM sucrose containing 1 mM ethylenediaminetetraacetic acid. Subcellular fractions were prepared by centrifugation of the homogenates at 35,000 g for 5 min. The first sediment was resuspended in the original volume and stored at −25°C. The "postmitochondrial" supernatant was centrifuged at 74,000 g for 31.5 min; the sediment was then resuspended and submitted again to centrifugation at 74,000 g for another 31.5 min. The sediment ("washed microsomes") was resuspended to a preparation equivalent to 2 g wet wt of liver per milliliter of suspension.

Bilirubin (18) and *p*-nitrophenol (19) UDP-glucuronosyltransferase activities were usually determined in digitonin-activated liver preparations; the protein concentration was measured in untreated preparations (20). Optimal activation by digitonin was checked by incubating the samples with various concentrations of digitonin kept on melting ice for 1 h. Highest activations were found by incubating a digitonin solution of 10 mg/ml with 10% (wt/vol) homogenate or with 25% (wt/vol) postmitochondrial supernatant or with 30% (wt/vol) "first" sediment. For microsomes, a digitonin to protein ratio of 1:4 was used (18). The activation ratio was similar in preparations from male and female rats.

Statistics

Statistical analysis was performed by the Student's *t*-test. Results are expressed as mean values ± 1 SD.

Results

Sex Differences in Hepatic Uridine Diphosphate-Glucuronosyltransferase

Microsomal bilirubin UDP-glucuronosyltransferase activity in female rat liver was significantly higher than in male animals although only 27% of the total activity was recovered in this preparation in females vs. 46% in males (Table 1). This sex difference was far more pronounced in the first sediment obtained and in total homogenates. The first sediment comprises nuclei, nuclear envelopes, mitochondria, lysosomes, and heavy fractions of the endoplasmic reticulum but not the light particles nor the cytosol. The volume of the first sediment obtained at 35,000 g was always ~7% larger in female than in male rats. These data would suggest that female rat liver contains more bilirubin UDP-glucuronosyltransferase and that it is dispersed over more heavy subfractions or that larger particles are formed during homogenization. Because of these observations, we used homogenates for further investigations. In contrast to the present results and to those of Wong (11), in some studies (8–10) with "microsomal" preparations marked differences were not observed between male and female rats, presumably because of the loss of most of the enzyme during preparation. Indeed for most microsomal fractions, recovery of transferase varies between 19% (18) and 27%–40% (present data). Furthermore age, weight, and protein content of the liver is frequently not specified. Strebel and Odell (10) found over 95% of enzyme activity in microsomes prepared in 150 mM KCl. This high recovery, however, was calculated relative to the supernatant obtained at 10,000 g for 10 min. No data are given on the loss of enzyme activity during this first step. Interestingly the same authors obtained a significantly lower recovery (69%) when microsomes were isolated in 250 mM sucrose containing 1 mM ethylenediaminetetraacetic acid.

Transferase activities of male and female rat liver at the age of 2, 4, 6, 8, and 10 wk are given in Figure 1. Activities were similar in both sexes on the second and the fourth week of life; they were 1.75-fold higher on the fourth than on the second week (20.1 ±

Table 1. Sex Differences in Bilirubin Uridine Diphosphate-Glucuronosyltransferase Activity of Rat Liver

Preparation	Bilirubin UDP-glucuronosyltransferase activity (*nmol/h · mg of protein*)		Calculated recovery as % of total activity per gram of liver		Proteins (*mg of liver*)	
	Male liver	Female liver	Male	Female	Male liver	Female liver
Homogenate	13.7 ± 1.2	28.4 ± 2.3[b]	100	100	209 ± 6	211 ± 4
First sediment	16.7 ± 3.2	44.7 ± 4.7[b]	57	69	108 ± 19	94 ± 16
Postmitochondrial supernatant	13.6 ± 1.9	13.3 ± 2.5	47	24	105 ± 6	109 ± 6
Microsomes	63.5 ± 6.6	76.5 ± 6.8[a]	46	27	22.4 ± 2.5	21.6 ± 2.6

Specific bilirubin uridine diphosphate-glucuronosyltransferase activity in different subcellular fractions from 6 male and 6 female rat livers are given. [a] $p < 0.01$. [b] $p < 0.001$.

1.6 nmol conjugated per hour per milligram of protein vs. 11.5 ± 2.6; $p < 0.001$). At the age of 8 and 10 wk transferase activity of female rat liver was nearly twofold compared with that of male liver (28.4 ± 4.3 vs. 15.8 ± 1.1, $p < 0.001$ and 26.4 ± 1.1 vs. 14.8 ± 1.1, $p < 0.001$). This difference results both from a further increase of activity in the females and from a decrease in the males. It contrasts with the conjugation of *p*-nitrophenol, as male rat liver had higher activities than female rat liver (1200 ± 126 vs. 912 ± 66 nmol/h · mg of protein; n = 3). The time of differentiation coincides with the average onset of puberty in the rat, taking place in both sexes around the seventh or eighth week of life (21). The larger spreading of values on the sixth week of life, without any clear separation between the two sexes, could be the result of individual differences in the onset of puberty. This transitory situation of the enzyme before puberty should be taken into account when choosing the age of rats for experiments on bilirubin metabolism.

Effects of Orchiectomy and of Administration of Testosterone

To check whether steroid androgens have a depressing effect on bilirubin conjugation, male rats aged 7 wk were submitted to orchiectomy and some received testosterone. Orchiectomy significantly decreased both body and liver weight; these two parameters remained normal in the orchiectomized rats kept on replacement therapy with testosterone (Table 2). Orchiectomy caused a significant rise in transferase activity, when compared with sham-operated animals (16.5 ± 0.9 vs. 13.5 ± 1.0, $p < 0.001$), whereas administration of testosterone decreased the enzyme activity to values observed in normal male rats. Serum testosterone levels were below the limit of detectability by radioimmunoassay (<30 pmol/L) in the castrated animals. In the orchiectomized animals treated with testosterone, plasma testosterone levels were 31 ± 9 nmol/L, comparable to values in normal males (range 14–35 nmol/L; and Reference 22).

Effect of Ovariectomy and of Administration of Estradiol or Progesterone

To investigate the increase of transferase activity observed in female rat liver after puberty, 7-wk-old female rats were ovariectomized; some were given estradiol or progesterone or a combination of the two. Ovariectomy significantly increased body weight (Table 2). Estradiol alone or in association with progesterone prevented this increase in body weight. Progesterone alone had no effect on body or liver weight. Only the administration of estra-

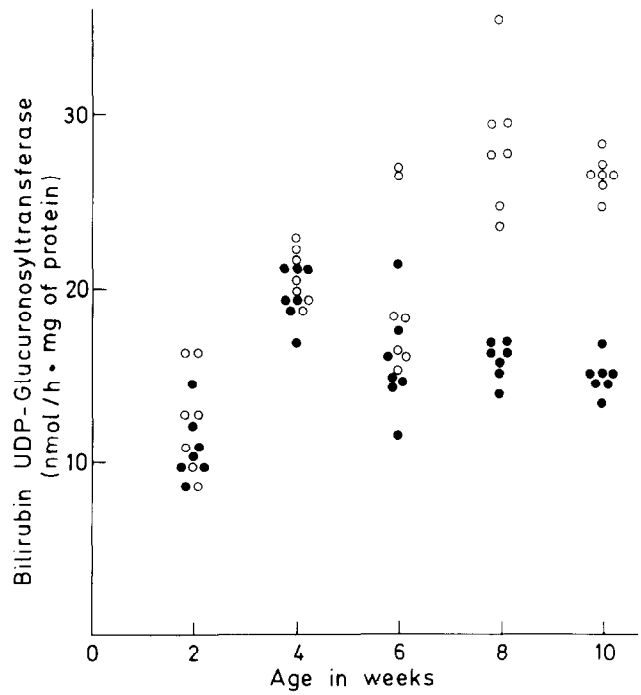

Figure 1. Sexual differentiation of rat hepatic bilirubin uridine diphosphate glucuronosyltransferase, assayed in digitonin-activated liver homogenates. Each group consisted of 7 male (*closed symbols*) and 7 female animals (*open symbols*).

Table 2. Effect of Gonadectomy or Steroid Replacement on Body and Liver Weight and on Hepatic Bilirubin Uridine Diphosphate-Glucuronosyltransferase

Sex	Treatment	Body weight (g)	Liver weight (g)	Enzyme activity (*nmol/h per mg of protein*)
Male	Sham-operation	264 ± 18	9.9 ± 1.0	13.5 ± 1.0
Male	Orchiectomy	206 ± 6[d]	8.0 ± 0.5[c]	16.5 ± 0.9[d]
Male	Orchiectomy + testosterone	233 ± 15[e]	9.0 ± 0.7	14.4 ± 0.7[f]
Female	Sham-operation	158 ± 12[a]	5.8 ± 0.5[a]	29.0 ± 3.4[a]
Female	Ovariectomy	188 ± 14[e]	5.6 ± 0.4	21.0 ± 1.5[d]
Female	Ovariectomy + estradiol	158 ± 6[e]	5.7 ± 0.2	21.4 ± 1.1[d]
Female	Ovariectomy + progesterone	189 ± 4[e]	5.7 ± 0.4	25.8 ± 1.8[g]
Female	Ovariectomy + estradiol + progesterone	165 ± 6	6.4 ± 0.4[b,e]	31.2 ± 2.3[g]

All operations were performed on groups of 6 rats aged 7 wk. Steroid administration was started on the day of operation and continued for 21 days, as described in Materials and Methods. Differences are indicated as follows: [a] $p < 0.001$ (different from male rats); [b] $p < 0.05$, [c] $p < 0.01$, [d] $p < 0.001$ (different from sham-operated rats of the same sex); [e] $p < 0.05$, [f] $p < 0.01$, [g] $p < 0.001$ (different from gonadectomized untreated rats of the same sex).

diol associated with progesterone increased the liver weight significantly.

Hepatic bilirubin UDP-glucuronosyltransferase was significantly lower in ovariectomized rats when compared with sham-operated controls (21.0 ± 1.5 vs. 29.0 ± 3.4, $p < 0.001$). Administration of estradiol to ovariectomized animals had no effect, but administration of progesterone significantly increased the activity to 25.8 ± 1.9 ($p < 0.001$); this value was still higher than in orchiectomized males (Table 2). Administration of the two steroids in combination led to enzyme levels similar to those noted in normal females (Table 2). In gonadectomized females, serum estradiol was 58 ± 10 pmol/L and serum progesterone was 79 ± 35 nmol/L. The combined treatment given to ovariectomized rats led to plasma estradiol levels of 209 ± 37 pmol/L, values entirely within the range found in normal females (158 ± 80 pmol/L). Combined treatment also led to progesterone levels of 204 ± 10 nmol/L; these values are similar to those obtained in the luteal phase of normal female rats.

Looking for a more practical way of administration, progesterone was then given as a long-acting preparation in three different dosages to ovariectomized animals in combination with a subcutaneous implant of estradiol. Administration of a total dose of 25 mg of Proluton-dépôt (Schering) gave values most comparable to those obtained with daily administration of 5 mg of progesterone and to values of normal female rats (Table 3). A clear-cut dose-related effect was not present among these three groups, although the group treated with the highest dose had significantly higher values than the group treated with the lowest dose.

Table 3. Effect of Different Doses of Progestogens on Hepatic Bilirubin Uridine Diphosphate-Glucuronosyltransferase

Treatment	Enzyme activity (*nmol/h · mg of protein*)
Ovariectomy + estradiol + vehicle only	20.3 ± 2.9
Ovariectomy + estradiol + proluton (25 mg)	36.9 ± 5.9[a]
Ovariectomy + estradiol + proluton (50 mg)	42.1 ± 11.3[a]
Ovariectomy + estradiol + proluton (100 mg)	50.8 ± 9.2[a,b]

Ovariectomies were performed in groups of 5 rats aged 7 wk. On the same day, estradiol was administered by a subcutaneous implant (see Materials and Methods). Proluton-dépôt (Schering) was given by subcutaneous injection, which was repeated after 10 days. Animals were killed after 21 days of treatment. Differences are indicated as follows: [a] $p < 0.001$ (different from gonadectomized rats not subjected to hormonal treatment); [b] $p < 0.05$ (different from rats treated with the lowest dose of progestogens).

Discussion

A sex difference has already been demonstrated in the rat for the hepatic glucuronoconjugation of a number of endogenous and exogenous substrates. Significantly higher rates have been reported in males for the glucuronoconjugation of *o*-aminophenol (5) and *p*-nitrophenol (13, 23, and the present study) whereas higher rates have been found in females for estrone, estradiol, and testosterone (24) and, in the present study, for bilirubin. It appears therefore that the influence of sex and of gonadectomy on glucuronoconjugation differs according to the substrate tested. Although it is not possible at present to clearly define groups of substrates whose conjugation would be affected in the same way, it is

of interest to note that *o*-aminophenol and *p*-nitrophenol belong to the so-called late fetal group and the other substrates to the early neonatal group (25).

Uridine diphosphate-glucuronosyltransferase is an enzyme system embedded in the membranes of the endoplasmic reticulum; its activity can be critically influenced by changes in its environment (26). It has been proposed that part of the hormonal control of the transferase could be mediated by alterations of the membrane environment. This could be suspected from changes in the activation characteristics of the enzyme in vitro (9,26). However, identical activation rates were obtained in the present study when liver preparations from male and female rats were assayed with and without activation by various amounts of digitonin. Bilirubin UDP-glucuronosyltransferase activities obtained with untreated liver were approximately sevenfold lower than with preparations activated with the optimal digitonin concentration (see Materials and Methods). As the entire activation curves were identical, it appears unlikely that the sex difference of bilirubin UDP-glucuronosyltransferase is mediated by a change in its membrane environment.

The sex difference in glucuronosyltransferase activity clearly developed at puberty. However, gonadectomy decreased but did not abolish (Table 2) the difference. This suggests that it is not only determined by postpubertal gonadal secretion of steroids. Extragonadal steroids might interact although they are quantitatively rather unimportant, or neonatal imprinting might exist (23). This phenomenon has been demonstrated for hepatic cortisol-metabolizing enzymes (27,28). Enzymes of animals of both sexes will develop into a feminine pattern except when androgens presumably secreted by the testicles during the neonatal period would program some areas of the brain into a male type of metabolism (28). Replacement therapy with testosterone or estro-progestogens, yielding normal plasma steroid levels, resulted in restoration of enzyme activities documented in normal male or female rats. In males, the fall in glucuronosyltransferase activity registered after puberty seems thus to be due to secretion of androgens by the mature gonads. In contrast with the present findings on bilirubin conjugation, previous studies reported an enhancement of the hepatic glucuronoconjugation of *o*-aminophenol by testosterone (5,7), whereas neither orchiectomy nor administration of testosterone affected the activity of testosterone-glucuronosyltransferase (24). In the females, changes observed at puberty are likely to be the result of gonadal secretion of female sex steroids. Previous studies only investigated the effect of estrogens. Estradiol was found to inhibit *o*-aminophenol glucuronoconjugation in male rat liver (5) and to increase estrone but not testosterone conjugation in ovariectomized rats (24). However, in the present study, whereas estradiol alone exerted no effect on bilirubin conjugation, progesterone produced a dose-dependent increase, which was even more pronounced when given in combination with estradiol. Progesterone is a known inducer of the hepatic smooth endoplasmic reticulum and of cytochrome P_{450} (29). The metabolic actions of steroid hormones are mediated by binding to intracytoplasmatic receptors, and the concentration of these receptors is of critical importance in determining the magnitude of metabolic response. Estradiol increases the concentration of receptors for progesterone in rat uterine tissue, thus amplifying the response to the latter hormone (30). It is not yet known if such a mechanism is also present in the liver, but, if so, this could easily explain the potentiating action of estradiol on the inducing effect of progesterone for bilirubin glucuronoconjugation.

The bilirubin UDP-glucuronosyltransferase activity of female rat liver found in excess of that of male liver was mainly recovered in fractions sedimenting at a lower gravity on ultracentrifugation. This is compatible either with enrichment in rough endoplasmic reticulum or with changes in the composition of the endoplasmic reticulum yielding more large vesicles during homogenization, as observed in different conditions by others (31). These possibilities are currently being investigated by combined ultracentrifugation and electron microscopic investigations. A sex difference for bilirubin UDP-glucuronosyltransferase was not observed in the postmitochondrial supernatant that still contains the cytosol. This is important as recent studies documented higher concentrations of the fatty acid binding (or A/Z) protein in female than in male cytosol (32). This protein binds bilirubin but it is not yet clear if it is involved in bilirubin transport in vivo.

The presently documented sexual differentiation at puberty of bilirubin glucuronoconjugation in rat liver seems thus mediated both by an inhibitory effect of testosterone and an enhancing effect of estro-progestogens. These in vitro observations are also evident in vivo, as female rats can conjugate and excrete a load of bilirubin more efficiently than male rats (33). A linear relationship was found between the transferase activity and the maximal bilirubin output in bile in normal and gonadectomized male and female rats.

If the same mechanisms are operative in humans, higher serum bilirubin levels in postpubertal males as compared with females might be the result of a lower conjugating capacity. Indeed their bilirubin clearance rate expressed per kilogram of body weight is lower (34) whereas the production rate was equal (34). Similar mechanisms might also explain why

Gilbert's syndrome, resulting from bilirubin UDP-glucuronosyltransferase deficiency, becomes apparent mainly after puberty in males.

References

1. Werner M, Tolls RE, Hultin JV, Mellecker J. Influence of sex and age on the normal range of eleven serum constituents. Z Klin Chem Klin Biochem 1970;8:105–15.
2. Owens D, Evans J. Population studies in Gilbert's syndrome. J Med Genet 1975;12:152–6.
3. Dutton GJ. The biosynthesis of glucuronides. In: Dutton GJ, ed. Glucuronic acid, free and combined. New York: Academic, 1966:186–299.
4. Fevery J, Van de Vijver M, Michiels R, Heirwegh KPM. Comparison in different species of biliary bilirubin-IX conjugates with the activities of hepatic and renal bilirubin-IX uridine diphosphate glycosyltransferases. Biochem J 1977; 164:737–46.
5. Inscoe JK, Axelrod J. Some factors affecting glucuronide formation in vitro. J Pharmacol Exp Ther 1980;129:128–31.
6. Hartiali KJW, Pulkkinnen MO. Studies on the possible role of sex hormones on the change in conjugation capacity at various ages in rat. Ann Acad Sci Fenn 1964;106(Suppl 12):1–8.
7. Künzel B, Müller-Oerlinghausen B. Wirkung von Testosteron und einem Anti-Androgen (Cyproteronacetat) auf die Glukuronidbildung in der Rattenleber. Pharmak Exp Path 1969; 262:112–23.
8. Halac E, Sicignano C. Re-evaluation of the influence of sex, age, pregnancy and phenobarbital on the activity of UDP-glucuronyltransferase in rat liver. J Lab Clin Med 1969;73:677–85.
9. Winsnes A. Age and sex dependent variability of the activation characteristics of UDP-glucuronyltransferase in vitro. Biochem Pharmacol 1971;20:1249–58.
10. Strebel L, Odell GB. Bilirubin uridine diphosphate-glucuronyltransferase in rat liver microsomes: genetic variation and maturation. Pediatr Res 1971;5:548–59.
11. Wong KP. Bilirubin glucosyl- and glucuronyltransferases. A comparative study and the effects of drugs. Biochem Pharmacol 1972;21:1485–91.
12. Hietanen E. Effect of sex and castration on hepatic and intestinal activity of drug-metabolizing enzymes. Pharmacology 1974;12:84–9.
13. Chabra RS, Fouts R. Sex differences in the metabolism of xenobiotics by extrahepatic tissue in rats. Drug Metabol Disp 1974;2:375–9.
14. Waynforth HB. Experimental and surgical technique in the rat. London: Academic, 1980:160–299.
15. Lax ER, Rüdiger G, Schriefers H. The hormonal regulation of hepatic microsomal 11β-hydroxysteroid dehydrogenase activity in the rat. Acta Endocrinol (Copenh) 1978;89:352–8.
16. Koninckx PR, Heyns W, Verhoeven G, et al. Biochemical characterisation of peritoneal fluid in women during the menstrual cycle. J Clin Endocrinol Metab 1980;51:1239–44.
17. Verhoeven G, Koninckx PR, De Moor P. Androgen and progestogen production in cultured interstitial cells derived from immature rat testis. J Steroid Biochem 1982;17:319–30.
18. Heirwegh KPM, Van de Vijver M, Fevery J. Assay and properties of digitonin-activated bilirubin uridine diphosphate glucuronosyl-transferase from rat liver. Biochem J 1972;129:605–18.
19. Zakim D, Vessey DA. Techniques for the characterization of UDP-glucuronyltransferase, glucose-6-phosphatase and other tightly-bound microsomal enzymes. Methods Biochem Anal 1973;21:1–37.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
21. Baker DEJ. Reproduction and breeding. In: Baker HJ, Lindsey JR, Weisbroth SH, eds. The laboratory rat. New York: Academic, 1979:153–68.
22. Lafourie M, Aussel C, Castelli D, Krebs B, Stora C. Sex steroid hormone levels and testicular activity during hepatocarcinogenesis by N-2-fluorenylacetamide. Res Comm Chem Pathol Pharmacol 1982;37:49–63.
23. Lamartiniere CA, Dieringer CS, Kita E, Lucier GW. Altered sexual differentiation of hepatic uridine diphosphate glucuronyltransferase by neonatal hormone treatment in rat. Biochem J 1979;180:313–8.
24. Rao GS, Haueter G, Rao ML, Breuer H. Steroid glucuronyltransferases of rat liver. Biochem J 1977;162:545–56.
25. Wishart GJ. Functional heterogeneity of UDP-glucuronosyltransferease as indicated by its differential development and inducibility by glucocorticoids. Biochem J 1978;174:485–9.
26. Dutton GJ. Control of UDP-glucuronosyltransferase activity. Biochem Pharmacol 1975;24:1835–41.
27. De Moor P, Denef C. The "puberty" of rat liver. Feminine pattern of cortisol metabolism in male rats castrated at birth. Endocrinology 1968;83:791–8.
28. Gustafsson JA, Skett P. Pituitary control of hepatic steroid metabolism. Acta Biol Med Germ 1979;38:307–20.
29. Jones AL, Emaus JB. The effect of progesterone administration on hepatic endoplasmic reticulum: an electron microscopic and biochemical study. In: Salhanick HA, Kipnes D, van de Wiele RL, eds. New York: Plenum, 1969:68–85.
30. Milgram E, Beaulieu EE. Progesterone in uterus and plasma. I. Binding in rat uterus 105,000 g supernatant. Endocrinology 1970;87:276–87.
31. Massey ED, Butler WH. Zonal changes in the rat liver after chronic administration of phenobarbitone: an ultrastructural, morphometric and biochemical correlation. Chem Biol Interac 1979;24:329–44.
32. Ockner RK, Burnett DA, Lysenko N, Manning JA. Sex differences in long chain fatty acid utilization and fatty acid binding protein concentration in rat liver. J Clin Invest 1979;64:172–81.
33. Muraca M, De Groote J, Fevery J. Sex differences of hepatic conjugation of bilirubin determine its maximal biliary excretion in non-anaesthetized male and female rats. Clin Sci 1983;64:85–90.
34. Berk PD, Howe RB, Blommer JR, Berlin NI. Studies of bilirubin kinetics in normal adults. J Clin Invest 1969;48:2176–90.