Liver Regeneration in the Presence of High Levels of Steroid Hormones. (32277)

LEON L. GERSHBEIN
Department of Biochemistry, Northwest Institute for Medical Research, Chicago

The liver undergoes various changes in composition and metabolism during pregnancy and lactation and the extent of its regeneration is increased in partially hepatectomized pregnant rats(1,2). In an approach to the mechanism of the latter behavior, a study was undertaken of the effect of high levels of estrogen and progesterone on liver weight restoration in operated rats, the action of androgens also being screened in animals of either sex.

Liver removal has been investigated in relation to uterine water uptake induced by estrogen(3-8) and a stimulation of liver regeneration in partially hepatectomized animals as such or with prior ovariectomy has been claimed in the presence of such agents (9-11). The interconversion of estrogenic hormones has been demonstrated by liver perfusion technique(12) and in partially hepatectomized rats(13); in the latter, urinary estrogen excretion was elevated. Bengmark and Olsson(14) noted an inhibition of hepatic fatty infiltration in male rats administered a long-acting testosterone preparation prior to partial hepatectomy but weight and glutamic pyruvic transaminase of the liver were not affected, in agreement with earlier findings of Rindi and coworkers(15). A rather low order of activity for androgens on the regenerative process is also evident from other reports(9, 16-18).

Materials and methods. The steroids and diethylstilbestrol of good quality were obtained from several commercial sources, including potassium estrone sulfate (KES, Penick), norethanedrolone (17α-ethyl-19-nortestosterone; Nilevar®, Searle) and methandrostenolone (1-dehydro-17α-methyltestosterone; Dianabol®, Ciba). The agents were triturated on a weight basis with Rockland rat meal or injected subcutaneously in peanut oil, the controls receiving the oil alone. KES was used in saline solution.

Holtzman rats of either sex were partially hepatectomized under ether anesthesia by the technique of Higgins and Anderson(19) and the liver portions dried to constant weight at 100°C. The animals were administered diets and water *ad lib* for a period of 10.5 days after which time they were sacrificed and the entire livers removed; the duration was 5 days for one group. Small portions of fresh tissue were preserved for histological study. The extent of hepatic regeneration or the increment was calculated from the dry liver weights by subtracting the amount excised at surgery multiplied by the factor 0.46 from the liver mass at necropsy(20). Animals suffering losses in excess of about 12-15% of the initial body weight were excluded from the calculations.

Results. Liver increment and body weight data together with standard errors and Fisher *t* values for adult rats of either sex injected daily for the first 7 days and sacrificed 10.5 days following surgery, appear in Table I. The volume of peanut oil or the oil mixture was 0.50-0.80 ml. Weight losses in males were high with estradiol benzoate and quite moderate with KES; half of the animals on norethanedrolone (over-all dosage; 2800 mg/kg) succumbed over the treatment period. The last series was instituted to discern possible extreme hepatic alterations. The effects of mixtures: testosterone propionate + estradiol benzoate, testosterone propionate + progesterone and estradiol benzoate + progesterone were also ascertained. In one series, the injections were made 3, 24 and 48 hours postoperatively, the animals then being sacrificed on the fifth day. With the latter, over 30% of the rats on progesterone alone and in a mixture with estradiol benzoate and testosterone propionate succumbed just prior to or after the third injection. Table I also presents findings for immature males and females (age: 41 days) administered testosterone propionate at a total dosage of 2800 mg/kg.

The pertinent data for males on diets con-

TABLE I. Liver Regeneration in Rats Administered Steroids Subcutaneously.†

Agent (mg/rat/day; over-all: mg/kg)	No. of rats	Body wt, g ± S.E. Initial	Body wt, g ± S.E. At necropsy	Liver increment, g ± S.E.	t
Group 1-1D (Males)					
Controls	13	270 ± 8.6	266 ± 8.6	2.025 ± .100	
Progesterone (25; 665)	10	262 ± 5.6	266 ± 7.4	2.209 ±1.123	1.17
Group 2-9D (Males)					
Controls	14	308 ±10.0	312 ± 6.6	1.941 ± .056	
Testosterone propionate (120; 2670)	11	330 ±13.0	300 ± 8.8	2.033 ±1.134	.47
Methyltestosterone (120; 2750)	10	325 ±15.7	285 ± 9.4	2.030 ± .134	.70
Progesterone (80; 1850)	10	306 ± 9.1	300 ± 7.4	2.498 ± .138	3.87**
Estradiol benzoate (0.10; 23)	10	300 ± 8.5	267 ± 7.1	1.859 ± .068	.93
Estradiol benzoate (0.50; 12.5)	9	309 ± 8.3	260 ± 4.2	1.809 ± .088	1.35
Methandrostenolone (15; 340)	12	320 ±11.5	295 ± 7.3	2.453 ± .095	4.83**
Norethanedrolone (25; 560)	12	320 ±11.4	307 ± 8.4	2.520 ± .076	6.25**
Group 3-11D (Males)					
Controls	13	248 ± 6.0	250 ± 8.2	1.805 ± .089	
Norethanedrolone (100; 2800)	8	251 ± 4.5	246 ± 6.3	2.210 ± .130	2.68*
Group 4-5R (Males)‡					
Controls	16	296 ± 4.5	298 ± 7.0	2.088 ± .078	
KES (2.0; 51)	12	290 ± 4.9	262 ± 5.2	1.844 ± .076	1.83
Group 6-15D (Females)					
Controls	14	198 ± 3.7	219 ± 4.2	1.676 ± .073	
Progesterone (5.0; 167)	13	197 ± 3.0	220 ± 3.6	1.792 ± .121	.94
Progesterone (20; 680)	12	195 ± 2.8	217 ± 6.3	2.118 ± .157	2.64*
Estradiol benzoate (0.35; 13)	11	199 ± 3.0	189 ± 5.8	1.673 ± .096	.03
Diethylstilbestrol (15; 544)	8	203 ± 3.9	182 ± 5.1	1.696 ± .068	.19
Estrone (2.0; 71)	10	202 ± 3.9	194 ± 4.2	1.692 ± .100	.13
Norethanedrolone (15; 507)	10	198 ± 3.0	215 ± 8.0	2.113 ± .138	3.01**
Methandrostenolone (10; 337)	11	198 ± 2.7	217 ± 5.5	1.624 ± .110	.40
Testosterone propionate (80; 2700)	10	203 ± 3.2	213 ± 4.9	2.064 ± .100	3.23**
Group 7-16D (Females)					
Controls	15	219 ± 2.1	242 ±10.7	2.071 ± .109	
Methyltestosterone (80; 2500)	15	220 ± 1.7	228 ± 3.8	2.048 ± .073	.17
Progesterone (20; 585)	13	223 ± 2.1	254 ± 3.0	2.544 ± .048	3.79**
Progesterone (20; 630) + estradiol benzoate (0.35; 11)	13	220 ± 2.4	215 ± 2.9	2.209 ± .088	.97
Group 8-17D (Females)					
Controls	19	199 ± 1.4	227 ± 1.8	1.535 ± .049	
Testosterone propionate (80; 2700)	18	199 ± 1.1	214 ± 3.3	1.889 ± .067	4.27**
Testosterone propionate (80; 2700) + estradiol benzoate (0.35; 12)	18	197 ± 1.1	206 ± 3.1	1.835 ± .053	4.17**
Testosterone propionate (80; 2590) + progesterone (20; 640)	17	199 ± 1.4	232 ± 2.3	2.342 ± .057	9.72**
Group 9-18D (Males)					
Controls	12	147 ± 2.4	169 ± 5.9	1.722 ± .074	
Testosterone propionate (60; 2800)	9	144 ± 2.7	160 ± 5.0	1.650 ± .065	.71
Group 10-19D (Females)					
Controls	14	141 ± 2.1	152 ± 4.4	1.792 ± .100	
Testosterone propionate (60; 2800)	15	144 ± 1.7	159 ± 2.9	1.916 ± .051	1.13
Group 11-22D (Females)					
Controls	14	207 ± 3.1	207 ± 4.8	1.440 ± .061	
Testosterone propionate (80; 1150)	15	209 ± 2.9	208 ± 3.3	1.403 ± .064	.42
Progesterone (20; 285)	16	211 ± 1.6	215 ± 3.0	1.591 ± .064	1.72
Progesterone (20; 285) + testosterone propionate (80; 1150)	8	207 ± 1.9	212 ± 4.0	1.718 ± .085	3.81**

TABLE I (continued)

Agent (mg/rat/day; over-all: mg/kg)	No. of rats	Body wt, g ± S.E. Initial	At necropsy	Liver increment, g ± S.E.	t
Progesterone (20; 285) + estradiol benzoate (0.35; 5)	11	212 ± 1.8	199 ± 3.9	1.396 ± .046	.54

† Injected daily for the first 7 days post-operative, the duration being 10.5 days, except for Group 11-22D. For the latter, injections were made after 3, 24 and 48 hr and the animals were sacrificed on the fifth day.

‡ Aqueous solution; .30 ml daily.

* P <.02. ** P <.01.

taining norethanedrolone (0.20 and 0.50%), methandrostenolone (0.50%), progesterone (0.60%) and testosterone propionate (0.75%) and for females on the latter two rations in addition to testosterone (0.40%) and methyltestosterone (0.75%) are shown in Table II. A diet containing testosterone propionate (0.75%) are shown in Table II. A diet containing testosterone prepionate (0.75%) + progesterone (0.60%) was also fed to males. The consumption of diets containing diethylstilbestrol (0.040-0.20%) by either sex was so poor that very few data could be salvaged. Weight losses were small with norethanedrolone at 0.20% but more extensive with this steroid and methandrostenolone, each incorporated at 0.50%. The anesthetic action of progesterone was noted in animals administered the higher levels.

Microscopic examination of hematoxylin and eosin-stained liver sections from the treated animals disclosed no outstanding

TABLE II. Liver Weight Restoration in Adult Rats Fed Steroid-Containing Diets (Duration: 10.5 Days).

Diet (g %)	No. of rats	Body wt, g ± S.E. Initial	At necropsy	Liver increment, g ± S.E.	t
Group 12-5G (Males)					
Controls	14	271 ± 7.3	292 ± 8.5	2.090 ± .114	
Norethanedrolone (.50%)	8	260 ± 8.1	227 ± 7.7	2.198 ± .077	.67
Methandrostenolone (.50%)	8	263 ± 7.1	231 ± 6.6	1.750 ± .138	1.85
Group 13-33D (Males)					
Controls	15	281 ± 5.0	307 ± 7.7	2.146 ± .078	
Progesterone (.60%)	13	281 ± 5.5	277 ± 5.3	2.971 ± .134	5.50**
Group 14-2E (Males)					
Controls	13	277 ± 7.3	286 ± 9.1	2.117 ± .082	
Testosterone propionate (.75%)	10	283 ± 8.5	270 ± 8.4	2.164 ± .092	.39
Group 15-5E (Males)					
Controls	13	272 ± 8.8	275 ± 9.9	2.072 ± .070	
Testosterone propionate (.75%) + progesterone (.60%)	10	262 ± 8.3	248 ±10.2	2.852 ± .133	5.57**
Group 16-6E (Females)					
Controls	15	236 ± 2.5	241 ± 4.3	1.707 ± .099	
Progesterone (.60%)	11	233 ± 3.5	238 ± 7.0	2.044 ± .114	2.46*
Testosterone (.40%)	13	231 ± 1.5	245 ± 2.8	1.794 ± .074	.70
Group 17-8E (Females)					
Controls	15	233 ± 1.7	247 ± 2.0	1.895 ± .095	
Testosterone propionate (.75%)	13	234 ± 1.8	240 ± 4.5	2.472 ± .078	4.61**
Group 19-11E (Females)					
Controls	18	205 ± 1.5	224 ± 2.3	1.633 ± .073	
Testosterone propionate (.75%)	11	204 ± 2.1	220 ± 4.6	2.140 ± .081	4.50**
Methyltestosterone (.75%)	11	203 ± 3.1	184 ± 3.8	1.607 ± .081	.23

* P <.05. ** P <.01.

changes as compared to the respective controls.

Discussion. In the present study employing high injectable levels of hormonal agents, estradiol benzoate, estrone, KES and diethylstilbestrol were without effect on the extent of liver regeneration employing adult rats of both sexes as was the case with testosterone propionate and methyltestosterone in males. In this regard, an earlier report demonstrated that except for a depression with vitamin D_2, liver weight restoration in males was not influenced by high dosages of cholesterol and bile acids(21). However, liver regeneration was stimulated in the adult female injected with testosterone propionate at 2700 mg/kg, an effect which did not extend to immature rats of either sex nor to adult females on methyltestosterone (2500 mg/kg; Table I). Injection of the anabolic steroids, methandrostenolone (340 mg/kg) and norethanedrolone (560 mg/kg), accelerated the extent of liver regeneration in males but only the latter agent was effective in females. Body weights were far better maintained by female rats administered the androgenic hormones than by males. Diets containing methandrostenolone (0.50%), norethanedrolone (0.50%) or testosterone propionate (0.75%) were without effect on the rate of regeneration in males but the last ration proved stimulatory to the adult female (Table II). However, this effect did not obtain at a somewhat lower level employing testosterone at 0.40%, (or as based on propionate: 0.48%) and as in the injection series, methyltestosterone (0.75%) was without action in the female.

The subcutaneous administration of progesterone at an over-all level of 1850 mg/kg proved stimulatory to the regenerating liver of adult males but the increase in increment at 665 mg/kg was not statistically significant. On the other hand, the female was more sensitive to the action of the exogenous steroid even at a dosage of 680 mg/kg but the lower level (167 mg/kg) was ineffective. The feeding of a ration containing progesterone supplemented at 0.60% also accelerated liver regeneration in adults of either sex.

The findings with animals injected with mixtures of the steroids are of great interest. In the adult female, estradiol benzoate (11 mg/kg) which by itself had no effect on the regenerating liver, when administered together with progesterone, negated the action of the latter (Group 7-16D; Table I). The change in the increment lacked statistical significance as compared to the controls but was definitely lower than that produced by progesterone alone (t: 3.32; $p<0.01$). Estradiol benzoate (11 mg/kg) did not abolish the effect of testosterone propionate (2700 mg/kg), but a mixture of this androgen at a comparable dosage with progesterone (640 mg/kg) caused an even greater stimulatory action (Group 8-17D). Although a comparable mixture was not injected into the male, the feeding of a diet containing progesterone (0.60%) + testosterone propionate (0.75%) accelerated regeneration as with progesterone alone (Groups 13-33D and 15-5E; Table II).

To amplify further the effect of stimultaneously administered progesterone and testosterone propionate and to circumvent more maximalized regeneration occurring with the above observation period, adult females were injected with each steroid individually and in combination at 3, 24 and 48 hours postoperatively and sacrificed on the fifth day; the injection of progesterone + estradiol benzoate was also included (Group 11-22D; Table I). An elevated toxicity was encountered in this series with some of the agents due to the shorter period between the first injection and surgery and possibly predicated by greater damage to the liver, a key organ in steroid metabolism and detoxification. As compared to the controls, only the series on progesterone (285 mg/kg) + testosterone propionate (1150 mg/kg) produced a significant increase in liver increment. These data further attest to the requirement of massive levels of the various steroids for an effect. It might be pointed out that the increment for the series on progesterone + estradiol benzoate was definitely lower than that of progesterone treatment alone (t: 2.24; $p<0.05$), again demonstrating a possible antagonism. A synergistic action is noted between progesterone and testosterone in the female. Furthermore, as comparably high levels of the testosterone propionate (2800 mg/kg) do not in-

fluence the extent of regeneration in the immature animal of either sex, the effect of this androgen in the adult female might be potentiated by endogenous progesterone. The mechanism is undoubtedly more complex, considering that estrogen which might negate the contribution of endogeneous progesterone did not influence the accelerating action of testosterone propionate given concurrently. In this conjunction, the testosterone-binding ability of human serum has been shown to be especially high in advanced pregnancy(22).

The sex differences noted in liver weight restoration in relation to subcutaneously injected progesterone and androgens might not be too surprising. Definite variations on a sex basis have been described for liver and these are reviewed by Bucher(23). Kelly and coworkers(24) suggest that hepatic DNA which is higher and more variable in the female mouse is a result of the response of the DNA-containing reticuloendothelial cells to estrogen. Such cells also display differences in radio-gold uptake(25). It is quite likely as based on the present liver regenerative criteria that the Kupfer cells might also be markedly affected by progesterone as such and in conjunction with estrogen or testosterone, unequivocal studies of which have not been advanced to date. Considering the elevated level of endogenous progesterone and the diminution of folliculoid attending pregnancy, the former steroid contributes greatly to the increase in liver weight restoration observed in partially hepatectomized gravid animals.

Summary. Massive dosages of steroids have been administered subcutaneously and by diet to partially hepatectomized rats of either sex and the extent of liver regeneration ascertianed over a period of 10.5 days. Progesterone accelerated this process in both sexes and as tested in adult females, the effect could be antagonized by estrogen. High levels of injected anabolic steroids, methandrostenolone and norethanedrolone, stimulated regeneration in males but only the latter proved effective in the female under the specified conditions; in males, both agents were ineffective in diets at levels of 0.50%. Testosterone propionate, but not methyltestosterone, accelerated liver regeneration in the adult female, an effect which may involve potentiation by progesterone. Estrogen had no direct influence on the stimulation engendered by testosterone.

1. Gershbein, L. L., Proc. Soc. Exp. Biol. & Med., 1958, v99, 716.
2. Paschkis, K. E., Cantarow, A., Cancer Res., 1958, v18, 1060.
3. Szego, C. M., Roberts, S., Endocrinology, 1945, v36, 104.
4. Roberts, S., Szego, C. M., ibid., 1947, v40, 73.
5. Szego, C. M., Roberts, S., J. Biol. Chem., 1949, v178, 827.
6. ———, Recent Progr. Hormone Research, 1953, v8, 419.
7. Botticelli, C. R., Hisaw, F. L., Jr., Proc. Soc. Exp. Biol. & Med., 1959, v100, 591.
8. Daniels, J. R., Kalman, S. M., J. Endocrinol., 1963, v28, 73.
9. Ber, A., Endokrynologia Polska, 1957, v8, 228.
10. Leonardi, R., Rigano, A., Minerva Ginecol., 1959, v11, 833.
11. Leonardi, R., ibid., 1961, v13, 1174.
12. Schiller, J., Pincus, G., Science, 1943, v98, 410.
13. ———, Endocrinology, 1944, v34, 203.
14. Bengmark, S., Olsson, R., Acta Chir. Scand., 1964, v127, 93.
15. Rindi, G., Allegri, A., Rizzolini, G. F., Arch. Fisiol., 1955, v55, 152.
16. Allegri, A., Boll. Soc. Med. (Pavia), 1951, v65, 113.
17. Taleisnik, S., Rev. Soc. Arg. Biol., 1953, v29, 87.
18. Seki, K., Osaka Daigaku Igaku Zasshi, 1959, v11, 5427.
19. Higgins, G. M., Anderson, R. M., Arch. Path., 1931, v12, 186.
20. Gershbein, L. L., Labow, J. A., Am. J. Physiol., 1953, v173, 55.
21. Gershbein, L. L., Int. Z. Vitaminforsch., 1965, v35, 264.
22. Pearlman, W. H., Crépy, O., J. Biol. Chem., 1967, v242, 182.
23. Bucher, N. L. R., Int. Rev. Cytol., 1963, v15, 245.
24. Kelly, L. S., Dobson, E. L., Finney, C. R., Hirsch, J. D., Am. J. Physiol., 1960, v198, 1134.
25. Stern, K., Duwelius, A., Proc. Soc. Exp. Biol. & Med., 1959, v100, 546.

Received March 16, 1967. P.S.E.B.M., 1967, v125.