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Effect of Sex Hormones on Ethanol Induced Fatty Infiltration of Liver in Rats. (23697)

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It has been previously reported that either chronic or acute ethanol intoxication causes lipid to accumulate in the liver of rats(1,2), and that under the same conditions of acute ethanol intoxication, female rats show a more severe fatty infiltration of the liver than do males(2). The experiments reported below were therefore carried out in order to determine the effect of male and female sex hormones on alcohol-induced fatty infiltration of the liver.

Procedure. Eight groups of male and 6 groups of female albino rats, weighing 70-80 g at the start, were maintained on Purina dog chow pellets *ad libitum* for periods of either 4 or 6 weeks. Three of the groups of males and 2 of the groups of females were castrated at the beginning of the experiment. During these periods of time, two groups of intact males received daily intramuscular injections (0.1 ml) of estradiol benzoate in sesame oil which was diluted with Wesson oil, and one such group received daily injections of 0.1 ml of Wesson oil alone. The dose of estradiol benzoate was 0.005 mg/day during the first 2 weeks, and 0.01 mg/day during the last 4 weeks. Testosterone propionate in oil (0.1 ml) was similarly administered to 1 group of castrated males, the dose being 0.1 mg/day during the first 2 weeks and 0.2 mg/day during the last 2 weeks, and to 2 groups of intact females, the dose being 0.1 mg/day during the first week, and increased weekly to 0.4 mg/day. The remaining groups received no injections. All animals weighed approximately 200 g at the end of the 4- or 6-week periods, when they were killed by decapitation. The longer period was required only for those rats receiving estrogen, because the hormone, in the dosage given, was found to exert a small inhibitory effect on the rate of gain of weight. At the end of the 4 or 6 weeks, the rats in 8 of the groups received a single intoxicating dose of ethanol (4.0 ml of a 50% solution by volume) by stomach tube, while those in the remaining groups received a single dose of glucose, calorically equivalent to the dose of alcohol, in the same volume of solution, and in the same manner. All food was then withdrawn, the female rats killed 18 hrs and the male rats 16 hrs thereafter. The livers were immediately removed, samples of liver frozen and subsequently analyzed for total lipids as already described(2). The 18- and 16-hour intervals subsequent to stomach tubing were chosen because it was previously found that female and male rats show peak depositions of liver lipid, subsequent to ethanol intoxication, at these times(2). The seminal vesicles were also removed from 6, and testes from 3 of the groups of males at the time of killing. The fluid was expressed from the seminal vesicles, and these organs as well as the testes weighed on a Roller Smith balance.

TABLE I. Effect of Sex Hormones on Fatty Infiltration of Liver of Ethanol Intoxicated Rats.

Group	No. rats	Ethanol or glucose	Hormone or oil (daily dose)	Total liver lipid, %	P	Sem. vesicles, mg/100 g body wt	P	Testes, mg/100 g body wt	P
				Intact male rats					
1	11	Glucose		5.5 ± .11		116.3 ± 5.97		1173 ±53.7	
2	11	"	.005-.01 mg estradiol benzoate in oil	5.8 ± .17	P >.1 (1 & 2)*	60.4 ± 3.69	P <.01 (1 & 5)	173.4± 5.53	P <.01 (1 & 5)
3	13	Ethanol		7.7 ± .16	P <.01 (1 & 3)				
4	8	"	.1 ml in oil	7.6 ± .33	P >.1 (3 & 4)				
5	9	"	.005-.01 mg estradiol benzoate in oil	7.7 ± .26	P >.1 (3 & 5)	56.9 ± 3.16	P <.01 (1 & 5)	180.1±15.4	P <.01 (1 & 5)
				Castrated male rats					
6	11	Glucose		6.2 ± .17	P <.01 (1 & 6)	9.2 ± .37 (30 rats)	P <.01 (1 & 6)		
7	19	Ethanol		9.7 ± .37	P <.01 (3 & 7) P >.1 (7 & 11)				
8	10	"	.1-.2 mg testosterone propionate in oil	6.7 ± .18	P <.01 (3 & 8)	220.8 ± 13.9	P <.01 (1 & 8)		
				Intact female rats					
9	10	Glucose		6.7 ± .21	P <.01 (9 & 11)				
10	12	"	.1-.4 mg testosterone propionate in oil	5.8 ± .12	P <.01 (9 & 10)				
11	11	Ethanol		10.7 ± .50	P <.01 (11 & 12)				
12	12	"	*Idem*	7.5 ± .13	P >.1 (12 & 13)				
				Ovariectomized female rats					
13	8	Glucose		5.6 ± .09	P <.01 (9 & 13) P <.01 (13 & 14)				
14	24	Ethanol		10.2 ± .29	P >.1 (11 & 14)				

* Numbers in parentheses refer to groups.

Results. (A) *Alcohol-induced fatty infiltration of the liver.* The experimental data are summarized in Table I. Prior administration of estradiol to intact male rats did not alter the ethanol-induced fatty infiltration of the liver (Group 3 *vs.* 5) although the weights of seminal vesicles and testes indicated that a potent dose of estrogen had been administered (Group 1 *vs.* 2 and 5). There was also no effect of ovariectomy of female rats on liver lipid accumulation (Group 11 *vs.* 14).

In contrast to this lack of effect of estrogen, androgen exerted a marked influence on the amount of liver lipid present after ethanol administration. Thus, castrated male rats (Group 7) accumulated much more lipid than did intact males (Groups 3 and 4). In fact, castrated males showed as high a liver lipid concentration as did females, there being no statistically significant difference between the 2 groups (Group 7 *vs.* 11). When castrated males received testosterone however (Group 8), this increase in liver lipid concentration over that in intact males, disappeared. Indeed, even less lipid accumulated in the livers of the testosterone treated, castrated male rats

than in the livers of intact males (Groups 3 and 8). This was probably due to the administration of excessively large quantities of testosterone, as indicated by the significantly greater weights of the seminal vesicles of castrated males receiving testosterone, than of intact males receiving no hormone (Group 8 *vs.* 1). In accordance with the above observations on male rats, it was found that intact female rats receiving testosterone showed a significant decrease in ethanol-induced accumulation of liver lipid. Thus, these animals showed the same liver lipid concentrations as did intact males, in response to ethanol administration (Groups 12 and 3).

Injection of oil vehicle itself did not influence the level of liver lipid (Group 4 *vs.* 3).

(B) *Liver lipid in non-alcoholic rats.* Since it was thought that castration, or the administration of hormone, might affect liver lipid values, apart from ethanol intoxication, controls were run in which isocaloric quantities of glucose were administered in place of ethanol, to operated and hormone treated rats. These procedures did, in some instances, alter the normal liver lipid concentrations, but only to a small degree, and not enough to mask the larger effects on the fatty infiltration of the liver promoted by ethanol intoxication. Thus the administration of testosterone to intact females receiving glucose produced a small but significant decrease in liver lipid concentration (Group 10 *vs.* 9). Castrated male rats receiving glucose, on the other hand, had small but significantly higher liver lipid values than did glucose-fed intact rats (Group 6 *vs.* 1). Thus the effect of male sex hormones on the control liver lipid values was qualitatively the same as that on the liver lipid values following ethanol intoxication, but quantitatively much smaller.

Administration of estrogen to males exerted no influence on the liver values of the glucose controls (Group 2 *vs.* 1). This too was similar to the lack of effect on liver lipids after ethanol intoxication. On the other hand, ovariectomy of female rats did produce a small but significant decrease in liver lipid concentration of the controls (Group 13 *vs.* 9).

Discussion. It may be considered possible that the apparent lack of effect of estradiol on the ethanol-induced fatty livers was in reality a resultant of two opposed forces: (1) a direct fatty liver promoting effect and (2) an indirect inhibitory effect (*via* the pituitary) on fatty liver production. Thus estradiol did have some inhibitory effect on rate of growth as well as on testicular and seminal vesicular weight (Group 1 *vs.* 2 or 5), presumably largely *via* pituitary inhibition, and it has been previously observed that hypophysectomy prevents ethanol-induced fatty infiltration of liver in rats(2). However, since animals receiving estrogen still had substantially larger quantities of testosterone available than did castrated rats, as seen from seminal vesicular weights (Groups 2 and 5 *vs.* 6); since administration of estradiol produced no change whatsoever in liver lipid concentration (Group 3 *vs.* 5); and since ovariectomy of female rats also produced no change in liver lipid after alcohol, the first and simpler explanation appears preferable.

Reports have appeared in the literature that procedures other than ethanol administration also result in a differential fatty infiltration of the liver between the sexes. Thus, more lipid has been found to accrue in the livers of female than of male rats after partial hepatectomy(3), the administration of diets low in protein and high in fat(4,5), and the administration of pituitary extracts(6). A lipotropic activity of male sex hormones would help to explain these phenomena also.

György and co-workers found, however, that in the case of fatty livers produced in rats by diets low in protein and high in fat, testosterone exerted no lipotropic effect, while the estrogens did exert such an effect(7,8). It was noted that the lipotropic action of estradiol benzoate was manifested only when methionine was also administered. On this same diet, female rats showed a greater increase in liver fat after ovariectomy, but castration had no effect on the fatty liver of male rats(9). These results are in contrast to our observations on ethanol-induced fatty livers. It is therefore possible that the particular effect of the sex hormones on liver lipid may vary with the mechanism that produces the fatty infiltration.

Summary. Female rats showed a more severe fatty infiltration of the liver than did male rats following ethanol intoxication. Administration of estradiol to male rats, or ovariectomy of female rats, prior to such intoxication, did not affect this fatty infiltration. On the other hand, administration of testosterone to female rats reduced the liver lipid concentrations to values similar to those obtained with males, while castration of males increased the liver lipid concentrations to those obtained with females. Castrated male rats, when treated with testosterone however, no longer showed liver lipid values that were higher than those of intact males. These results suggest that testosterone exerts a lipotropic action on ethanol-induced fatty infiltration of the livers in rats, while the estrogens do not influence this process.

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Diurnal Variation in Mouse and Rat Liver Sulfhydryl.* (23698)

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In the course of experiments performed for another purpose it was noted that otherwise untreated mice sacrificed in the afternoon exhibited significantly smaller liver non-protein sulfhydryl concentrations (NPSH) than did untreated mice sacrificed in the morning of the same day. This paper is concerned with an investigation of this phenomenon.

Methods. Liver extracts were secured by grinding the tissue with ice-cold 2% sulfosalicylic acid or 6% HPO_3, 19.3 ml/g, to give a 20-fold dilution of tissue NPSH. Filtered extracts were held in a refrigerator to time of analysis, always on the same day. The nitroprusside method of Grunert and Phillips(1) for estimation of NPSH was modified to assure adequate neutralization of HPO_3, to minimize effects of fading of the nitroprusside color, and to fit our equipment (Bausch and Lomb spectrophotometer, 1 inch cuvettes, wave length 525 mμ). The procedure employed was as follows: (a) for the sulfosalicylic acid extracts and solutions, 2.5 ml of the Grunert-Phillips carbonate-cyanide reagent and 2.5 ml of 2% sodium nitroprusside were placed in a large test tube; for the HPO_3 extracts and solutions, 3 ml of the carbonate-cyanide reagent and 2 ml of 2.5% Na nitroprusside were placed in a large test tube, (b) 2 ml of the NPSH containing extract or solution and 18 ml of saturated NaCl were placed in a cuvette, (c) with the cuvette in place, the spectrophotometer was adjusted to read ZERO optical density, (d) the contents of the test tube and cuvette were rapidly and thoroughly mixed and the test optical density reading obtained. In certain of the experiments (Table I), not only was NPSH of each extract estimated, but also glutathione (GSH) by the alloxan method of Lazarow(2). This method is one which Lazarow has stated is not entirely satisfactory for tissue extracts, although suitable for blood. Sulfosalicylic acid liver extracts could not be employed be-

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