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.

1. Mallov, S., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 246.

2. Mallov, S., Block, J. L., *Am. J. Physiol.*, 1956, v184, 29.

3. MacKay, E. M., Carne, H. O., PROC. SOC. EXP. BIOL. AND MED., 1938, v38, 131.

4. MacKay, E. M., *Am. J. Physiol.*, 1937, v119, 783.

5. ———, *ibid.*, 1937, v120, 361.

6. MacKay, E. M., Barnes, R. H., *ibid.*, 1937, v118, 525.

7. György, P., Rose, C. S., Shipley, R. A., *Arch. Biochem.*, 1947, v12, 125.

8. ———, *ibid.*, 1949, v22, 108.

9. Shipley, R. A., Chudzik, E. B., György, P., *ibid.*, 1948, v16, 301.

Received November 8, 1957. P.S.E.B.M., 1958, v97.

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

LYLE V. BECK, VIRGINIA D. RIECK AND BLENDINA DUNCAN†

Department of Physiology and Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

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-

* This research was supported in part by NIH Grant.

† We are indebted to Charlotte Batdorf for technical assistance.

TABLE I. Further Studies on Relation between Time of Day and Liver Sulfhydryl.

	Liver NPSH and GSH as GSH equiv., mg %, ± stand. error*							
	Untreated rats (Exp. 3)†		Untreated mice		Sham operated mice (Exp. 6 + Exp. 7)§		Adrenalectomized mice (Exp. 6 + Exp. 7)§	
Time of day	NPSH	GSH‡	NPSH Exp. 4	NPSH Exp. 5	NPSH	GSH	NPSH	GSH
8 A.M.	260 ± 6	208	284 ± 6	304 ± 8	352 ± 14	290 ± 12	259 ± 13	209 ± 15
12 noon	226 ± 7	199	303 ± 8	332 ± 12	274 ± 13	229 ± 9	280 ± 15	227 ± 9
4 P.M.	200 ± 7	184	219 ± 10	230 ± 12	268 ± 12	209 ± 8	248 ± 12	207 ± 12
8 "	179 ± 6	113	219 ± 11	201 ± 9	227 ± 13	170 ± 12	207 ± 8	146 ± 12
12 midnight	182 ± 7	160	224 ± 9	230 ± 8	261 ± 10	230 ± 9	251 ± 11	209 ± 11
4 A.M.	234 ± 7	211	283 ± 7	274 ± 9				

NPSH = non-protein sulfhydryl; GSH = glutathione.

* Each mean value is for analyses on 9 to 12 separate livers. † Rat wts, 140-180 g. ‡ One analysis only, on a pooled extract. § Data for Exp. 6 and 7 pooled.

cause of strong absorption at 305 mμ. Absorption by 6% HPO_3 was small. However, appreciable absorption at 305 mμ did occur for 6% HPO_3 liver extracts not containing alloxan. Hence the GSH values of Table I must be considered as being less reliable than the corresponding NPSH values. The mixture employed for securing a test optical density reading for a given extract was prepared as follows: (a) the original tissue extract was diluted 4, 5 or 10 times with 6% HPO_3, (b) 2 ml of this *diluted* extract were mixed with 3 ml of M/3 sodium phosphate buffer of pH 7.55, 2 ml of 0.1 N alloxan and 1 ml of 1.25 N NaOH (pH of this mixture about 7.45), and (c) *7 minutes later,* 2 ml of 1.0 N NaOH were added and the test optical density at 305 mμ secured immediately, using a Beckman DU spectrophotometer. The *blank* for the same extract was prepared in the same manner except that all alkali solutions were added before the alloxan, in order to prevent formation of the alloxan-glutathione complex, and the 7 minute waiting period was eliminated. CFW male mice, 20-26 g body weight, were employed. In Exp. 4 and 5 (Table I) the mice were held in an air-conditioned room at 25-27°C during and for 4 days before sacrifice. Wistar strain female rats were used, of body weights indicated under Results. Operations (sham or adrenalectomy) were performed on mice 4 days before the day of sacrifice. Due to the time required for these operations the sum total of the data of Exp. 6 and 7 (Table I) could be obtained only by performing 2 separate experiments, with resulting greater scatter of pooled data than typically occurs in a single experiment. The animal housing rooms had daylight exposure. Rats were held in open mesh wire cages, mice in transparent plastic cages with sawdust on the bottom. Food was available at all times and consisted of Rockland rat or mouse pellets. Food and water were replenished in the rat cages each morning, in the mouse cages when necessary. Animals were placed in clean cages a day or so before any given experiment. All of the animals to be sacrificed at a given time were placed in the same cage or set of cages, to minimize disturbance of animals not scheduled to be sacrificed at that time.

Results. Two experiments were performed in which control (untreated) animals were sacrificed at 1½ hour intervals over the period between about 9:30 a.m. and 4:30 p.m. (the maximum time spread over which control animals had been sacrificed in previous experiments). Male mice exhibited a progressive decrease in liver NPSH (expressed as mg %, GSH equivalent, ± standard error) from a high value of 296 ± 11 at 9:30 a.m. to a low value of 187 ± 14 at 4:30 p.m. ($p<0.001$). Female rats of weights 83 to 146 g exhibited a progressive decrease from a high value of 211 ± 8 at 9:30 a.m. to a low value of 134 ± 10 at 4:30 p.m. ($p<0.001$). It would appear very important, therefore, that any experiment performed to test for the effect of a procedure on mouse or rat liver NPSH or GSH should be performed in such a manner that control and test animals are sacrificed at as nearly the same time of day as possible. The data of Table I are for experiments in which

animals were sacrificed at 4-hour intervals over a 20- or 24-hour period. In these experiments maximal values for both liver NPSH and GSH occurred at 8 a.m. or 12 noon, minimal values at 8 p.m. The diurnal variation in GSH was sufficient to account for all of the diurnal variations in NPSH. These relations held for untreated, sham operated and adrenalectomized male mice, whether kept in an air-conditioned room or not; they also held for untreated female rats. Diurnal variations in liver NPSH and GSH were appreciably less for adrenalectomized than for either untreated or sham operated mice.

The unexpected finding of a considerable diurnal variation in liver NPSH and GSH of untreated mice and rats made it desirable to reexamine previously reported findings(3,4) that marked decreases in liver NPSH are induced by severe trauma or exposure to cold. An experiment was performed in which 9 test mice were subjected to tourniquet trauma(5) (both hind legs ligated 2 hours, sacrifice 2 hours after removal of ligatures). Five of the untreated control mice were sacrificed immediately before and 4 immediately after sacrifice of the traumatized mice. The following liver sulfhydryl values, expressed as mg %, GSH equivalent, ± standard error, were obtained: Control mice — NPSH = 261 ± 9, GSH = 222 ± 7; traumatized mice — NPSH = 142 ± 5, GSH = 135 ± 4. In this experiment very much lower liver sulfhydryl values were found for traumatized than for non-traumatized mice, although the time of day at which sacrifice occurred was as nearly as possible the same for both groups of mice.

Discussion. From the literature(2) it would appear that synthesis of GSH occurs mainly in the liver. We have found(6) that dietary sulfur amino acid deficiency results in a far greater decrease in mouse liver NPSH than in the NPSH of any of the other tissues analyzed (kidney, spleen, heart, skeletal muscle). This indicates that the liver not only manufactures but also stores GSH until it is needed elsewhere. Diurnal variation in GSH storage in the liver might be due primarily to variation in (a) dietary intake of sulfur amino acids, or (b) passage of GSH from the liver. It is well known that mice and rats exhibit appreciably greater food intake during the night than during the day; such a dietary pattern might very well result in a gradual increase during the night in the amount of GSH stored in the liver, and a gradual dissipation of stored liver GSH during the day. Whatever the mechanism, the diurnal variation in mouse and rat liver NPSH and GSH is of such a magnitude as to arouse suspicion that some reports in the literature of experimentally induced changes in mouse or rat liver NPSH or GSH may actually reflect in large part diurnal variation in GSH.

Summary. Untreated, sham operated and adrenalectomized male mice and Wistar Strain female rats all exhibited statistically significant diurnal variations in liver NPSH and GSH. High NPSH and GSH values came at 8 a.m. or 12 noon, low at 8 p.m.

1. Grunert, R. R., Phillips, P. H., *Arch. Biochem.,* 1951, v30, 217.

2. *Glutathione, A Symposium,* 1954, Academic Press, Inc., N. Y.

3. Beck. L. V., Linkenheimer, W., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 291.

4. Beck, L. V., Linkenheimer, W., Marraccini, A., *ibid.,* 1954, v86, 823.

5. Rosenthal, S. M., *U. S. Pub. Health Rep.,* 1943, v58, 1429.

6. Beck, L. V., Bianconi, A. M., *J. Nutrition,* 1956, v60, 197.

Received November 8, 1957. P.S.E.B.M., 1958, v97.