Biochemical Pharmacology, Vol. 34, No. 3, pp. 369–372, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

EFFECT OF CASTRATION ON THE TURNOVER OF RAT LIVER ALCOHOL DEHYDROGENASE

ESTEBAN MEZEY* and JAMES J. POTTER
Department of Medicine of The Baltimore City Hospitals, and The Johns Hopkins University School of Medicine, Baltimore, MD, U.S.A.

(*Received* 26 *January* 1984; *accepted* 11 *June* 1984)

Abstract—Castration increased liver alcohol dehydrogenase activity and enzyme protein in male rats. The turnover of alcohol dehydrogenase determined from the decline in radioactivity present in immunoprecipitated enzyme after injection of $NaH^{14}CO_3$ was decreased after castration. The fractional rate of degradation (K_d) for the enzyme was $0.11 \cdot day^{-1}$ in the castrated as compared with $0.13 \cdot day^{-1}$ in the control animals ($P < 0.05$). The fractional rate of synthesis (K_s) of the enzyme was not affected by castration, while the absolute rate of synthesis was increased slightly. This study shows that a decrease in the rate of degradation is the principal cause for the increase in liver alcohol dehydrogenase following castration.

The activity of rat liver alcohol dehydrogenase is affected by a variety of hormones. Hypophysectomy [1], castration [2–4] and thyroidectomy [5] result in increases in liver alcohol dehydrogenase activity which are suppressed by growth hormone, testosterone and thyroid hormones respectively. Stress, which stimulates the hypothalamo–hypophyseal–adrenocortical axis and the sympathetic nervous system [6], but decreases circulating testosterone levels [7], also increases the activity of liver alcohol dehydrogenase [8]. The purpose of this study was to determine whether the increase in liver alcohol dehydrogenase following castration results from activation of the enzyme or an increase in enzyme protein due to changes in its rate of synthesis or degradation.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing between 150 and 170 g were obtained from The Charles River Breeding Laboratories (Wilmington, MA). The animals were placed in wire mesh cages in a room at a controlled temperature of 20° with light/dark cycles alternating every 12 hr, beginning at 7:00 a.m., and provided water and Purina chow *ad lib*. Twenty animals were castrated under ether anesthesia, while another twenty had a sham surgical procedure consisting of dissection of the scrotum and manipulation of the testes with a forceps. Ten days after surgery at 9:00 a.m. each of the animals received a single intraperitoneal injection of 1.0 mCi $NaH^{14}CO_3$ (55 mCi/mmole, Amersham Corp., Arlington Heights, IL) per 100 g of body weight. Four castrated and four sham-operated control animals were killed by a blow on the neck at 9:00 a.m. on each of days 1, 3, 5, 7 and 9 after the injection. The livers were removed, rinsed in 1.15% KCl, weighed, and homogenized in 3 vol. of 0.05 M Tris–HCl buffer, pH 8.4. The homogenate was centrifuged at 10,000 *g* for 10 min, and the resulting supernatant fraction was centrifuged at 39,000 *g* for 45 min. Part of the 39,000 *g* supernatant fraction was saved for determination of the incorporation of radioactivity into cytosolic protein. Alcohol dehydrogenase activity was determined in the 39,000 *g* supernatant fraction at 37° in 0.5 M Tris–HCl buffer, pH 7.2, by the method of Crow *et al.* [9]. One unit of enzyme activity is defined as the formation of 1 μmole of NADH per min. Cytosolic protein concentration was determined by the method of Lowry *et al.* [10] with bovine serum albumin used as a standard.

For the determination of the amount of radioactivity incorporated into liver alcohol dehydrogenase, the enzyme was precipitated from the 39,000 *g* supernatant fraction with antibody against rat liver alcohol dehydrogenase. This antibody was obtained from rabbits injected with rat liver alcohol dehydrogenase which had been purified to homogeneity as described previously [11]. The prepared antibody to rat liver alcohol dehydrogenase cross-reacted with mouse liver alcohol dehydrogenase but not with the horse or human liver enzyme. The amount of antibody needed to precipitate all the enzyme present in the supernatant fractions was determined in initial studies and, thereafter, a constant ratio was maintained between enzyme activity and the amount of antibody required to precipitate it. The immunoprecipitates were washed with phosphate-buffered saline, pH 7.4, and subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis [12]. The gels were fixed in a solution of 28% trichloroacetic acid and 8.4% sulfosalicylic acid, and stained with Coomassie brilliant blue R-250. The protein bands corresponding to the 40,000 molecular weight subunit of liver alcohol dehy-

* Address all correspondence to: Esteban Mezey, M.D., Baltimore City Hospitals, 4940 Eastern Ave., Baltimore, MD 21224, U.S.A.

drogenase were identified from SDS-polyacrylamide gel electrophoresis of purified horse liver alcohol dehydrogenase (Sigma Chemical Co., St. Louis, MO) which was included in each electrophoretic run. Alcohol dehydrogenase protein was determined as described by von Hungen *et al.* [13] by scanning the gels at 560 nm in a Gilford spectrophotometer with a linear transport attachment. Gels which contained known amounts of purified horse liver alcohol dehydrogenase protein were used as standards. The protein bands were sliced and incubated at 56° in Soluene 350 (Packard Instrument Co., Downers Grove, IL) for 12 hr [14] and then counted in Aquasol-2 Universal Cocktail (New England Nuclear Corp., Boston, MA). The fractional rates of degradation (K_d) and synthesis (K_s) were obtained from the slopes of the regression lines of the decrease in total and specific radioactivity respectively. The slopes and the standard errors of the slopes were calculated by linear regression from all the data points (N = 20 for each slope). Equality of the variances around the lines was tested using the F test. Estimate of differences of the slopes was performed using the slopes and standard errors of the slopes, and the levels of significance were determined by Student's *t* distribution [15]. The absolute rate of synthesis (V) was obtained by multiplying K_s by the total liver alcohol dehydrogenase activity.

For determination of radioactivity incorporated into cytosolic protein, 0.2 ml of the cytosolic fractions was precipitated with cold 10% trichloroacetic acid, and the precipitates were washed three times with cold 5% trichloroacetic acid, followed by incubation in Soluene 350 and counting as described above. Radioactivity was calculated as disintegrations per minute in cytosolic protein per total liver.

RESULTS

Castration had no effect on body weight or liver weight but resulted in a decrease in total cytosolic protein (Table 1). Both liver alcohol dehydrogenase activity and enzyme-protein were increased in the castrated as compared with the control animals (Table 2).

The turnover of total radioactivity in liver alcohol dehydrogenase was decreased in the castrated animals (Fig. 1). The fractional rate of degradation (K_d) for the castrated animals was $0.11 \pm 0.0063 \cdot day^{-1}$ as compared with a value of $0.13 \pm 0.0068 \cdot day^{-1}$ in control animals ($P < 0.05$). This corresponds to half-life ($T_{\frac{1}{2}}$) values of 6.3 and 5.3 days for the enzyme in castrated and control animals (Fig. 2). The fractional rate of synthesis (K_s) was $0.11 \cdot day^{-1}$ in both cases. The absolute rate of synthesis (V) was increased to 2.0 units/day in the castrated as compared with 1.6 units/day in the controls.

The turnover of total cytosolic protein was also decreased by castration (Fig. 3), but this decrease

Table 1. Effect of castration on body weight, liver weight and total cytosolic protein*

	Body wt (g)	Liver wt (g)	Cytosolic protein (mg/liver)
Control	170.0 ± 5.6	9.6 ± 0.5	535.1 ± 20.2
Castrated	174.0 ± 2.4	10.2 ± 0.4	467.0 ± 16.3†

* Values of body weight are those at the time of isotope injection, while values of liver weight and cytosolic protein were obtained at sacrifice. All values are means ± S.E. of twenty animals in each group.

† Significantly different ($P < 0.05$) from control.

Table 2. Effect of castration on liver alcohol dehydrogenase activity and enzyme-protein*

	Alcohol dehydrogenase	
	Activity (units/liver)	Protein (mg/liver)
Control	14.6 ± 0.69	10.5 ± 1.19
Castrated	18.9 ± 0.96†	16.0 ± 1.64‡

* Values are means ± S.E. of twenty animals in each group.

† Significantly different ($P < 0.05$) from control.

‡ Significantly different ($P < 0.02$) from control.

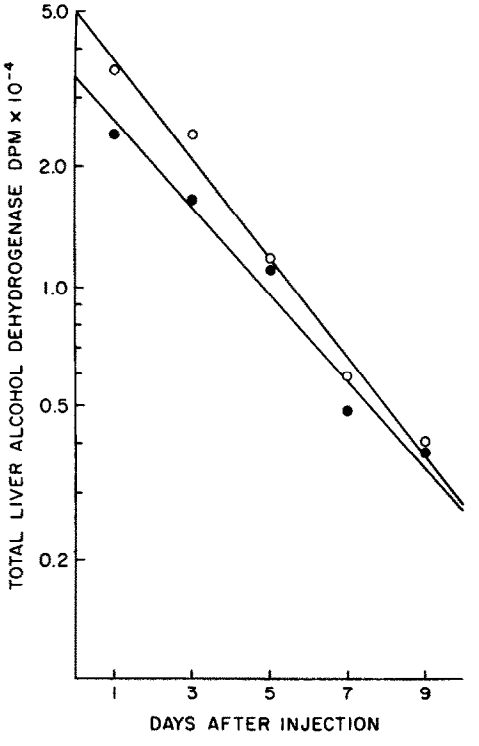

Fig. 1. Decrease in total radioactivity from liver alcohol dehydrogenase. The livers were labeled *in vivo* with a single intraperitoneal injection of $NaH^{14}CO_3$. Liver alcohol dehydrogenase was precipitated from the liver cytosol with antibody specific for rat liver alcohol dehydrogenase, and the radioactive enzyme was then isolated by SDS-polyacrylamide gel electrophoresis. Each point represents the mean total enzyme radioactivity in four control (○), and four castrated (●) animals killed at the indicated times. The correlation coefficients, r^2, for the linear least squares fit are 0.94 for the control and 0.93 for the castrated.

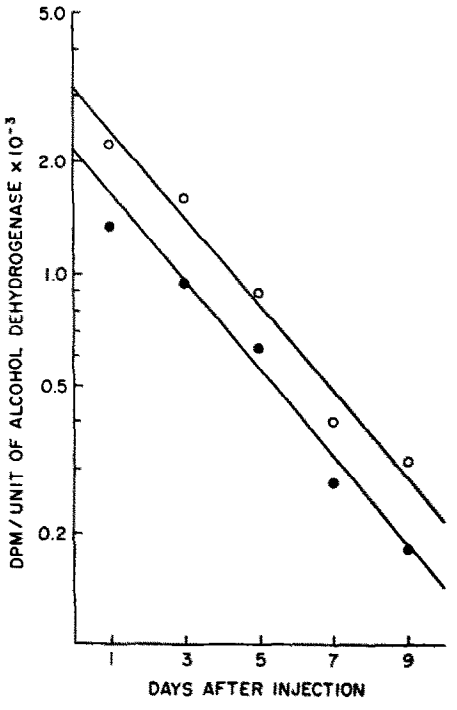

Fig. 2. Decrease of specific radioactivity from liver alcohol dehydrogenase. The livers were labeled *in vivo* with a single intraperitoneal injection of $NaH^{14}CO_3$. Liver alcohol dehydrogenase was isolated for radioactivity as described in Fig. 1. Each point represents the mean specific radioactivity for four control (○) and four castrated (●) animals killed at the indicated times. The correlation coefficients, r^2, for the linear least squares fit are 0.91 for the control and 0.94 for the castrated.

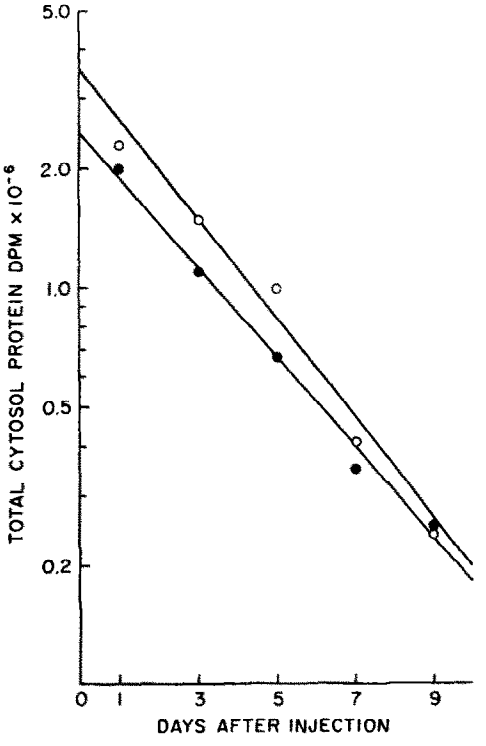

Fig. 3. Decrease of radioactivity from total cytosolic protein in livers labeled *in vivo* with a single intraperitoneal injection of $NaH^{14}CO_3$. Each point represents the mean radioactivity in total cytosolic protein of four control (○) and four castrated (●) animals. The correlation coefficients, r^2, for the linear least squares fit are 0.81 for both lines.

was not statistically significant. The fractional rate of degradation (K_d) of cytosolic protein was $0.11 \pm 0.011 \cdot day^{-1}$ in the castrated animals as compared with $0.13 \pm 0.014 \cdot day^{-1}$ in the controls ($P > 0.05$).

DISCUSSION

In agreement with prior studies [2–4], liver alcohol dehydrogenase activity was increased by castration in this study. Furthermore, the increase was found to be due to increased enzyme protein rather than to activation of the enzyme. A decrease in the rate of degradation appears to be the principlal cause of the increase in enzyme activity following castration. The fractional rate of degradation of $0.13 \cdot day^{-1}$ obtained for control animals is similar to the value of $0.14 \cdot day^{-1}$ reported by Guerri *et al.* [16] using the double isotope technique. Castration decreased the fractional rate of degradation in our study to $0.11 \cdot day^{-1}$. The decrease in K_d of $0.02 \cdot day^{-1}$ following castration would result in an increase in total liver alcohol dehydrogenase activity of 0.292 ($14.6 \text{ units} \cdot 0.02 \cdot day^{-1}$) units per day or approximately 4.26 units in 10 days. This value is very close to the actual difference of 4.30 units obtained between castrated and control animals in this study. Castration did not affect the fractional rate of synthesis. Nevertheless, an increase in the absolute rate of synthesis of the enzyme occurred after castration.

The fractional rate of degradation of hepatic cytosolic protein was identical to and decreased to the same extent as that of alcohol dehydrogenase following castration. However, in the case of cytosolic protein, the decreased degradation was not statistically significant and was associated with a decrease in cytosolic protein, suggesting that castration results in decreased cytosolic protein synthesis. Castration decreases DNA-dependent RNA polymerase in isolated rat liver nuclei, and this effect is reversed to normal after testosterone administration [17]. Also, testosterone increases protein synthesis by hepatocytes [18] and muscle [19] *in vitro*.

The mechanism for the decreased rate of degradation of liver alcohol dehydrogenase and cytosolic protein after castration is unknown. Decreased protein degradation is a major factor in the increase in liver protein found in other experimental situations such as after partial hepatectomy [20] or when protein-depleted animals are fed an adequate diet [21]. Parallel changes in the rate of proteolysis and in lysosomal size and fragility suggest that lysosomal enzymes participate in the degradation of endogenous protein [22]. Testosterone in *in vitro* experiments increases the release of lysosomal enzymes [23, 24], and such an effect may be decreased by castration.

Acknowledgement—This study was supported by Grant AA00626 from the United States Health Service.

REFERENCES

1. E. Mezey and J. J. Potter, *Endocrinology* **104**, 1667 (1979).
2. E. Mezey, J. J. Potter, S. M. Harmon and P. D. Tsitouras, *Biochem. Pharmac.* **29**, 3175 (1980).
3. G. Rachamin, J. A. MacDonald, S. Wahid, J. J. Clapp, J. M. Khanna and Y. Israel, *Biochem. J.* **186**, 483 (1980).
4. T. J. Cicero, J. D. Bernard and K. Newman, *J. Pharmac. exp. Ther.* **215**, 317 (1980).
5. E. Mezey and J. J. Potter, *Gastroenterology* **80**, 566 (1981).
6. S. M. McCann, L. Krulich, P. Illner, M. Quijada, K. Ajika and C. P. Fawcett, *Adv. exp. Med. Biol.* **33**, 157 (1971).
7. D. Repčeková and L. Mikulaj, *Hormone Res.* **8**, 51 (1977).
8. E. Mezey, J. J. Potter and R. Kvetňanský, *Biochem. Pharmac.* **28**, 657 (1979).
9. K. E. Crow, N. W. Cornell and R. L. Veech, *Alcoholism* **1**, 43 (1977).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
11. E. Mezey and J. J. Potter, *Archs Biochem. Biophys.* **255**, 787 (1983).
12. K. Weber and M. Osborn, *J. biol. Chem.* **244**, 4406 (1969).
13. K. von Hungen, R. C. Chin and C. F. Baxter, *Analyt. Biochem.* **128**, 398 (1983).
14. K. Smith and R. E. Ganschow, *J. biol. Chem.* **253**, 5437 (1978).
15. N. T. J. Bailey, *Statistical Methods in Biology*, p. 97. English University Press, London (1959).
16. C. Guerri, R. Wallace and S. Grisolia, *Eur. J. Biochem.* **86**, 581 (1978).
17. C. C. Widnell and J. R. Tata, *Biochem. J.* **98**, 621 (1966).
18. A. Bernelli-Zazzera, M. Bassi, R. Comolli and P. Lucchelli, *Nature, Lond.* **182**, 663 (1958).
19. C. B. Breuer and J. R. Florini, *Biochemistry* **4**, 1544 (1965).
20. O. A. Scornik and V. Botbol, *J. biol. Chem.* **251**, 2981 (1976).
21. R. D. Conde and O. A. Scornik, *Biochem. J.* **158**, 385 (1976).
22. G. E. Mortimore and C. M. Schworer, in *Protein Degradation in Health and Disease* (Eds. D. Evered and J. Whelan), Ciba Foundation Symposium 75 (New Series), p. 281. Excerpta Medica, Amsterdam (1980).
23. C. de Duve, R. Wattiaux and M. Wibo, *Biochem. Pharmac.* **9**, 97 (1962).
24. G. Weissman, *Biochem. Pharmac.* **14**, 525 (1965).