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EFFECTS OF CASTRATION AND TESTOSTERONE ADMINISTRATION ON RAT LIVER ALCOHOL DEHYDROGENASE ACTIVITY*

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Abstract—The effects of castration and testosterone administration on the activity of liver alcohol dehydrogenase and on the rate of ethanol elimination were determined in male Sprague–Dawley rats. Castration increased liver alcohol dehydrogenase activity. The total liver activity in castrated animals was 2.37 ± 0.229 (S.E.) mmoles/hr as compared with a value of 1.39 ± 0.125 mmoles/hr in sham-operated controls ($P < 0.01$). Testosterone administration partially suppressed the enhanced activity of liver alcohol dehydrogenase produced by castration. By contrast, in control animals testosterone administration resulted in a small paradoxical increase in liver alcohol dehydrogenase. The increase in the enzyme activity in castrated animals was associated with a parallel increase in the rate of ethanol elimination. Castrated and control animals showed decreases in free cytosolic and mitochondrial NAD^+/NADH ratios after ethanol administration. These observations suggest that testosterone (and probably other as yet unknown factors modified by castration) affects liver alcohol dehydrogenase activity, and that the total enzyme activity can be a principal limiting factor in ethanol elimination.

Liver alcohol dehydrogenase is the principal enzyme responsible for ethanol oxidation. The activity of liver alcohol dehydrogenase and the rate of ethanol elimination were found to be elevated after stress induced by immobilization [1]. Various forms of stress in man [2, 3] and immobilization stress in rats [4] result in a fall in plasma testosterone levels. Recently, castration was found to prevent the normal decreases in liver alcohol dehydrogenase activity and rate of ethanol elimination that occur in spontaneously hypertensive rats from 5 to 13 weeks of age. This effect of castration was reversed by concomitant administration of testosterone [5]. The purpose of the present study was to determine the effects of castration and the administration of testosterone on liver alcohol dehydrogenase and on the rate of ethanol elimination in Sprague–Dawley rats.

MATERIALS AND METHODS

Animals and treatments. Male Sprague–Dawley rats, initially weighing between 100 and 120 g (4–5 weeks of age), were kept in separate 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. They were provided water and Purina Chow *ad lib.* All the animals were killed between 10:00 and 11:00 a.m.

Twenty animals were castrated under ether anesthesia, while another twenty animals had a sham surgical procedure consisting of dissection of the scrotum and manipulation of the testes with a forceps. Starting 10 days after surgery, one-half of the castrated animals and one-half of the sham-operated control animals were given subcutaneous injections of testosterone propionate (Sigma Chemical Co., St. Louis, MO) at a dose of 0.5 mg/100 g body weight twice a day, while the remainder of the castrated and sham-operated animals were given isovolumetric amounts of corn oil (the testosterone vehicle). The injections of testosterone or vehicle were given for 6.5 days; the animals were killed 2 hr after the last injection.

Rates of ethanol disappearance from the blood were determined in another eight castrated and eight sham-operated control animals. The redox state of the liver was determined after the administration of saline or ethanol in additional groups of sixteen castrated and sixteen sham-operated control animals. All these determinations were done 10 days after surgery.

Determination of alcohol dehydrogenase. The animals were decapitated, and blood was collected in heparinized tubes for the determination of testosterone. The livers were removed, rinsed in 1.15% KCl, weighed, and homogenized in a Potter–Elvehjem homogenizer with 4 vol. of 0.05 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), sodium salt, pH 8.4, containing 0.33 mM dithiothreitol [6]. The homogenates were centrifuged at 106,000 *g* for 60 min at 4°. Alcohol dehydrogenase activity was determined in the resulting supernatant fraction at 37° by the method of Crow *et al.* [6]. The

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volume of the reaction mixture was 3 ml and consisted of 0.5 M Tris–HCl buffer (pH 7.2), 5 mM ethanol, 2.8 mM NAD^+, and 0.020 ml of the supernatant fraction of the liver homogenate. A blank reaction without ethanol was run in each case. The change in optical density at 340 nm was recorded for 5 min after the start of reaction. The alcohol dehydrogenase activities were then calculated from the molecular extinction coefficient of 6.22 $cm^2/\mu mole$ for NADH. Alcohol dehydrogenase was also determined in castrated and sham-operated animals in the reductive direction with 3.2 mM acetaldehyde as a substrate [7]. Protein concentration was determined by the method of Lowry *et al.* [8] with bovine serum albumin used as a standard.

Michaelis–Menten constants for ethanol and NAD^+ were calculated from Lineweaver–Burk plots obtained from determinations of alcohol dehydrogenase activity at nonsaturating ethanol and NAD^+ concentrations respectively.

Isoenzymes of alcohol dehydrogenase. Liver samples were homogenized in 2 vol. of water and then were centrifuged at 9000 *g* for 10 min. The resulting precipitate was discarded and the supernatant fraction was centrifuged at 106,000 *g* for 60 min. Starch gel electrophoresis of the 106,000 *g* supernatant fraction was carried out at pH 7.7 and 4° on horizontal 10.4% starch gel (Electrostarch Co., Madison, WI) for 20 hr as described by Smith *et al.* [9]. At the end of the electrophoresis the gels were stained for alcohol dehydrogenase activity by incubating them for 1 hr at 37° in 0.05 M Tris–HCl buffer (pH 8.6) containing (per 100 ml): 80 mg NAD^+, 40 mg nitro blue tetrazolium, 8 mg phenazine methosulfate, and 1.2 ml of 100% ethanol. Control gels were incubated in the absence of ethanol.

Rates of ethanol disappearance. Ethanol (2.0 g/kg of body weight) was injected intraperitoneally as a 10% solution (w/v) in water. Blood was obtained from the retro-orbital plexus of each animal, with a heparinized capillary tube, starting at 1 hr after ethanol administration and at 30-min intervals thereafter for 3 hr. After centrifugation at 2000 *g* for 10 min, the separated plasma samples were analyzed for ethanol concentration by gas–liquid chromatography [10]. Methanol was used as an internal standard for each sample. Ethanol concentrations in the plasma, when plotted against time, followed a linear function. The rate of ethanol disappearance from the plasma was obtained from the slope of the regression line calculated by the method of the least squares. The ethanol degradation rate, expressed in mmoles per kg of body weight per hr, was obtained by first calculating Widmark factor *r*, and then multiplying it by ten times the rate of ethanol disappearance from the blood.

Hepatic NAD^+/NADH ratio. Ethanol (2.0 g/kg of body weight) as a 10% solution (w/v) or an isovolumetric amount of saline was injected intraperitoneally into castrated and sham-operated animals. The animals were killed by a blow on the neck 2 hr after the injections. The livers were removed, freeze-clamped with aluminium plates as described by Wollenberger *et al.* [11], and dropped in liquid nitrogen. Less than 10 sec elapsed between the blow on the neck and the freezing of the liver. Thereafter, the frozen liver was pulverized in a precooled mortar with the addition of liquid nitrogen, transferred to a pre-weighed vessel containing 6% (w/v) perchloric acid and homogenized, and the weight of the liver recorded. Further treatment of the tissue consisted of centrifugation to separate precipitated protein and adjustment of the supernatant fraction to pH 6.0 with 2% KOH, followed by centrifugation to remove $KClO_4$ and treatment with Florisil (Floridin Co., Hancock, WV) as described by Williamson *et al.* [12]. The supernatant fraction obtained after centrifugation to remove Florisil was used for the determination of metabolites. Lactate was determined by the method of Hohorst [13] and pyruvate by the method of Bücher *et al.* [14]. β-Hydroxybutyrate was determined by the method of Mellanby and Williamson [15], and acetoacetate by the method of Williamson *et al.* [16]. The cytoplasmic free NAD^+/NADH ratio was calculated from the lactate dehydrogenase reaction [17], while the mitochondrial free NAD^+/NADH ratio was calculated from the β-hydroxybutyrate dehydrogenase reaction.

Radioimmunoassay of plasma testosterone. Blood was centrifuged at 2000 *g* for 10 min at 4°, and the separated plasma was stored frozen for the determination of testosterone levels. Radioimmunoassay for testosterone was carried out using Florisil for the separation of bound and free hormone (S. M. Harman, P. D. Tsitouras, M. A. Kowatch and A. Kowarski, manuscript submitted for publication). The antibody was supplied by Dr. A. Kowarski of the Johns Hopkins University School of Medicine. Assays were calibrated by use of pooled charcoal-extracted "blank" plasma to which known amounts

Table 1. Effects of castration and testosterone administration on body weight, liver weight, and cytosolic protein concentration*

	Body weight (g)	Liver weight (g)	Liver weight (g/100 g body wt)	Cytosolic protein (mg/g)
Control	214.2 ± 7.71	8.9 ± 0.14	4.2 ± 0.07	88.8 ± 1.93
Castrated	196·7 ± 4.71	8.0 ± 0.25	4.1 ± 0.07	90.8 ± 2.64
Control + testosterone	209.1 ± 8.61	8.9 ± 0.34	4.2 ± 0.09	87.2 ± 5.33
Castrated + testosterone	191.3 ± 2.59	7.9 ± 0.16	4.1 ± 0.08	86.7 ± 1.82

* All values are means ± S.E.M. of ten animals in each group.

of testosterone had been added. Intra-assay variance was 0.08, and inter-assay variance, 0.06, at 400 ng/dl concentration. Minimal sensitivity of the assay was 10 pg.

The results are expressed as means ± S.E.M. Statistical significance was determined by Student's *t*-test.

RESULTS

Castration and the administration of testosterone had no effect on body weight, liver weight, and the concentration of protein in the cytosol (Table 1). The plasma testosterone level in the castrated animals was 26.2 ± 2.22 ng/dl which is only slightly above the minimal sensitivity of the assay, and contrasts with a value of 340.3 ± 30.08 ng/dl obtained in the sham-operated animals ($P < 0.001$). Castrated and sham-operated animals that had received testosterone injections had plasma levels of testosterone of more than 2000 ng/dl at the time of killing. Castrated animals had the highest activity of liver alcohol dehydrogenase (Table 2); this activity was higher in the castrated than in the sham-operated control animals whether expressed per mg of protein, per g of wet liver weight, per kg of body weight, or total per rat. The increase in the enzyme activity was also demonstrated in the reductive direction with acetaldehyde as a substrate; the mean activity in the castrated animals was 12.52 ± 0.717 μmoles·mg^{-1}·hr^{-1} compared with 7.25 ± 0.581 μmoles·mg^{-1}·hr^{-1} in the control animals ($P < 0.001$).

Testosterone administration resulted in partial suppression of the enhanced activity of liver alcohol dehydrogenase produced by castration. This effect was statistically significant only when the enzyme activity was expressed per kg of body weight or as total per rat. By contrast, the administration of testosterone to control animals resulted in a paradoxical increase in liver alcohol dehydrogenase.

The K_m values of alcohol dehydrogenase for ethanol and for NAD^+ were not affected by castration or testosterone administration (Table 3).

Starch gel electrophoresis of the liver supernatant fraction revealed two bands of alcohol dehydrogenase activity migrating toward the cathode in castrated and sham-operated animals whether or not they had received testosterone.

Castrated animals had faster rates of elimination of ethanol than sham-operated control animals (Table 4).

The cytosolic and mitochondrial free NAD^+/NADH ratios were similar in control and castrated animals 2 hr after saline administration (Table 5). Two hr after ethanol administration the cytosolic and mitochondrial free NAD^+/NADH ratios decreased in both the control and castrated animals. The decrease in the cytosolic free NAD^+/NADH ratio was greater in the castrated than in the control animals ($P < 0.02$). whereas the decrease in the mitochondrial ratio was similar for both the castrated and control animals.

Table 2. Effects of castration and testosterone administration on liver alcohol dehydrogenase activity*

	Alcohol dehydrogenase			
	[μmoles · (mg protein)$^{-1}$ · hr^{-1}]	[μmoles · (g liver)$^{-1}$ · hr^{-1}]	[mmoles · (kg body wt)$^{-1}$ · hr^{-1}]	[mmoles · rat^{-1} · hr^{-1}]
Control	1.47 ± 0.080	159.6 ± 4.54	6.47 ± 0.485	1.39 ± 0.125
Castrated	2.60 ± 0.132†	274.6 ± 9.89†	12.04 ± 0.769†	2.37 ± 0.229‡
Control + testosterone	1.94 ± 0.177§	202.6 ± 8.94§	8.82 ± 0.704§	1.90 ± 0.190§
Castrated + testosterone	2.36 ± 0.151	197.8 ± 9.25	8.01 ± 0.319‖	1.52 ± 0.078‖

* All values are means ± S.E.M. of ten animals in each group.
† Significantly different from control, $P < 0.001$.
‡ Significantly different from control, $P < 0.01$.
§ Significantly different from control, $P < 0.05$.
‖ Significantly different from castrated, $P < 0.05$.

DISCUSSION

This study demonstrates that castration in male rats results in a marked increase in liver alcohol

Table 3. Michaelis–Menten constants of alcohol dehydrogenase after castration and testosterone administration

	K_m (M)	
	Ethanol	NAD^+
Control	4.6×10^{-4}	7.2×10^{-5}
Castrated	6.3×10^{-4}	6.2×10^{-5}
Control + testosterone	4.9×10^{-4}	4.2×10^{-5}
Castrated + testosterone	5.6×10^{-4}	3.7×10^{-5}

dehydrogenase activity, which is associated with an increase in the rate of ethanol elimination. These findings agree with studies in spontaneously hypertensive rats in which castration also resulted in increases in liver alcohol dehydrogenase activity and rate of ethanol elimination [5]. In spontaneously hypertensive rats there is a decrease in liver alcohol dehydrogenase and rate of ethanol elimination between 5 and 17 weeks of age which is prevented by castration [5]. In other rat species liver alcohol dehydrogenase activity increases from birth and reaches adult levels at 3 weeks [18]. The administration of testosterone completely suppressed the enhancing effect of castration on liver alcohol dehydrogenase in the spontaneously hypertensive rats. In this study testosterone administration was only partially effective in reversing the castration-induced increase in liver alcohol dehydrogenase. Furthermore, in this study testosterone produced a small paradoxical increase in liver alcohol dehydrogenase activity in sham-operated animals.

In previous studies we demonstrated increases in liver alcohol dehydrogenase during uremia produced by partial nephrectomy [19], after stress induced by immobilization [1], and after hypophysectomy [20]. Uremia in man [21], various forms of stress in man [2, 3], and immobilization stress in rats [4] all result in a fall in plasma testosterone levels. The effect of testosterone in partially suppressing the enhancing effect of castration on liver alcohol dehydrogenase activity while increasing the enzyme activity in intact animals is similar to the previously observed divergent effect of growth hormone in suppressing this enzyme activity in hypophysectomized animals, while increasing it in intact animals [20]. These observations suggest first, that alcohol dehydrogenase activity is regulated by more than one hormone and second, that the effect of the administration of one hormone on the enzyme activity may depend on its interaction with other hormones. Among the best known interactions are those between growth hormone and androgens. For example, the effect of growth hormone in decreasing ethylmorphine demethylation in normal male, but not female, rats appears to be due, in part, to an effect of growth hormone of antagonizing the stimulating action of testosterone on this microsomal enzyme [22]. Testosterone, in turn, is known to enhance maximum growth hormone levels in man after various stimuli such as hypoglycemia [23]. Furthermore, pituitary growth hormone concentrations are higher in the male than in the female rat; castration in the male decreases growth hormone to the level in the female, whereas administration of testosterone to the female increases growth hormone [24].

The changes in hepatic alcohol dehydrogenase activity after castration and the administration of testosterone were not associated with alterations in the Michaelis–Menten constants for ethanol or NAD^+, or with changes in isoenzyme pattern on starch gel electrophoresis. A similar lack of change in the Michaelis–Menten constants and electrophoretic mobility was found for the increased enzyme activity after experimental uremia [19], stress [1], and hypophysectomy [20]. The only difference is that two isoenzymes of liver alcohol dehydrogenase were demonstrated on starch gel electrophoresis in normal and experimental animals in this study as compared with one band in previous studies. This is due to an improvement in migration of the enzyme obtained by changing the type of starch used from that of Connaugh, Canada, to that of the Electro starch Co., Madison, WI, and decreasing its concentration in the gel from 14 to 10.4%. These changes were suggested by Dr. T. K. Li from the Indiana University School of Medicine, Indianapolis, IN, who first demonstrated two isoenzymes of rat liver alcohol dehydrogenase using this method (personal communication).

Factors that can determine the *in vivo* rate of ethanol oxidation by alcohol dehydrogenase are the total activity of liver alcohol dehydrogenase and the rate of reoxidation of NADH. The association between increases in liver alcohol dehydrogenase activity and increased rates of ethanol elimination after castration in this study, and after stress [1], and the parallel decrease in both after prolonged fasting [25] indicate that in these circumstances alcohol dehydrogenase activity is rate-limiting. The greater decrease in the cytosolic free NAD^+/NADH ratio in castrated than in control animals suggests that the rate of reoxidation of NADH can, in turn, become

Table 4. Effect of castration on rates of ethanol elimination *in vivo**

	Rates of ethanol elimination		
	[μmoles · (ml plasma)$^{-1}$ · hr^{-1}]	[mmoles · (kg body wt)$^{-1}$ · hr^{-1}]	[mmoles · rat^{-1} · hr^{-1}]
Control	8.78 ± 0.871	6.35 ± 0.506	1.03 ± 0.078
Castrated	12.97 ± 0.913†	9.57 ± 0.746†	1.54 ± 0.129†

* All values are means ± S.E.M. of eight animals in each group.
† Significantly different from control, $P < 0.01$.

Table 5. Effect of acute ethanol administration on the concentration of metabolites and the redox state in the freeze-clamped liver*

	Pyruvate (μmoles/g)	Lactate (μmoles/g)	$NAD^+/NADH$ cytosol	Acetoacetate (μmoles/g)	β-Hydroxybutyrate (μmoles/g)	$NAD^+/NADH$ mitochondria
Control						
Saline	0.093 ± 0.0139	0.807 ± 0.0913	1035.2 ± 115.76	0.025 ± 0.0052	0.146 ± 0.0233	4.34 ± 0.508
Ethanol	0.019 ± 0.0031†	0.762 ± 0.0876	255.8 ± 47.39†	0.025 ± 0.0020	0.280 ± 0.0823	2.54 ± 0.566‡
Castrated						
Saline	0.127 ± 0.0216	0.859 ± 0.1128	1308.5 ± 107.44	0.032 ± 0.0043	0.164 ± 0.0251	4.32 ± 0.538
Ethanol	0.015 ± 0.0027†	1.095 ± 0.1594	127.2 ± 13.76†§	0.022 ± 0.0057	0.218 ± 0.0760	1.94 ± 0.283‖

* All values are means ± S.E.M. of eight animals in each group.
† Significantly different from corresponding value after saline, $P < 0.001$.
‡ Significantly different from control after saline, $P < 0.05$.
§ Significantly different from control after ethanol, $P < 0.02$.
‖ Significantly different from castrated after saline, $P < 0.01$.

limiting at high ethanol oxidation rates resulting from the increased liver alcohol dehydrogenase activity. Higher activities of liver alcohol dehydrogenase obtained in this study, compared with our prior studies [1, 20], are the result of a change in the assay of the enzyme from that described by Bonnichsen and Brink [26] to one described by Crow *et al.* [6]. The principal determinants that result in a higher activity are stabilization of the enzyme in the homogenate by dithiothreitol, measurement of the activity at 37° instead of at 25–30°, the trapping of acetaldehyde by Tris buffer, and the activation of the enzyme by chloride ion [6].

In contrast to our present findings, there are many situations in which there is a lack of correlation between rate of ethanol elimination and the activity of liver alcohol dehydrogenase. These include an increased rate of ethanol elimination in association with a decreased enzyme activity after thyroxine administration [27], and normal rate of ethanol elimination in association with enhanced enzyme activity in experimental uremia [19]. Increases in the rate of ethanol elimination after ethanol administration have been associated with changes in liver alcohol dehydrogenase in only a few [28], and not most, studies [29, 30]. An increased reoxidation of NADH is a likely cause for increased ethanol elimination after thyroxine [27] and ethanol [31] administration. Stimuli to increased reoxidation of NADH, such as the administration of pyruvate [32] or fructose [33], enhance ethanol metabolism. Increases in the rate of ethanol elimination after thyroxine administration [27] and ethanol ingestion [31] were shown to occur in association with increased hepatic mitochondrial oxidative capacity in liver slices that was attributed to changes in phosphorylation potential resulting from increases in hepatic $(Na^+–K^+)$-stimulated ATPase. Increased oxygen consumption was also demonstrated in association with an increased rate of ethanol utilization by perfused livers after chronic ethanol feeding [34]. Other investigators, however, have failed to find increased mitochondrial oxidative capacity in association with increased rates of ethanol elimination in isolated hepatocytes after chronic ethanol administration [35, 36] or in liver slices after chronic stress [1]. Other possible mechanisms for increased reoxidation of NADH are increased transfer of NADH into mitochondria by either the malate-aspartate or α-glycerophosphate shuttle, or an increased reoxidation of NADH in the cytosol.

An increase in the microsomal oxidation of ethanol, which was not determined in this study, was demonstrated in association with increased rates of ethanol elimination after thyroxine administration [37] and ethanol feeding [38]. However, a lack of parallelism, following withdrawal from ethanol ingestion, between the rates of ethanol elimination, which fell rapidly, and the enzyme activity, which remained elevated for a prolonged time [39], suggests that increased microsomal oxidation of ethanol is unlikely to play a significant role in enhanced rates of ethanol elimination.

REFERENCES

1. E. Mezey, J. J. Potter and R. Kvetňanský, *Biochem. Pharmac.* **28**, 657 (1979).
2. B. W. Ellis, P. F. Evans, P. D. Phillips, A. F. Murray, H. S. Jacobs, V. H. T. James and H. A. F. Dudley, *J. Endocr.* **69**, 25P (1976).
3. L. E. Kreuz, R. M. Rose and J. R. Jennings, *Archs gen. Psychiat.* **26**, 479 (1972).
4. D. Repčeková and L. Mikulaj, *Hormone Res.* **8**, 51 (1977).
5. Y. Israel, J. M. Khanna, H. Orrego, G. Rachamin, S. Vahid, R. Britton and H. Kalant, *Drug Alcohol Depend.* **4**, 109 (1979).
6. K. E. Crow, N. W. Cornell and R. L. Veech, *Alcoholism: Clin. expl Res.* **1**, 43 (1977).
7. H. A. Krebs, R. A. Freeland, R. Hems and M. Stubbs, *Biochem. J.* **112**, 117 (1969).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
9. M. Smith, D. A. Hopkinson and H. Harris, *Ann. hum. Genet.* **35**, 243 (1972).
10. J. P. Payne, D. V. Foster, D. W. Hill and D. G. L. Wood, *Br. med. J.* **3**, 819 (1967).
11. A. Wollenberger, O. Ristau and G. Schoffa, *Pflügers Arch. ges. Physiol.* **270**, 399 (1960).
12. D. H. Williamson, P. Lund and H. A. Krebs, *Biochem. J.* **103**, 514 (1967).
13. H. J. Hohorst, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), 2nd Edn, p. 266. Academic Press, New York (1965).
14. T. Bücher, R. Czok, W. Lamprecht and E. Latzko, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), 2nd Edn, p. 253. Academic Press, New York (1965).

15. J. Mellanby and D. H. Williamson, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), 2nd Edn, p. 454. Academic Press, New York (1965).
16. D. H. Williamson, J. Mellanby and H. A. Krebs, *Biochem. J.* **82**, 90 (1962).
17. H. J. Hohorst, F. H. Kreutz and T. Bucher, *Biochem. Z.* **332**, 18 (1959).
18. N. C. R. Räiha, M. Koskinen and P. Pikkarainen, *Biochem. J.* **103**, 623 (1967).
19. E. Mezey, R. E. Vestal, J. J. Potter and J. W. Rowe, *J. Lab. clin. Med.* **86**, 931 (1975).
20. E. Mezey and J. J. Potter, *Endocrinology* **104**, 1667 (1979).
21. V. S. Lim and V. S. Fang, *J. clin. Endocr. Metab.* **43**, 1370 (1976).
22. R. E. Kramer, J. W. Greiner, R. C. Rumbaugh, T. D. Sweeney and H. D. Colby, *J. Pharmac. exp. Ther.* **204**, 247 (1978).
23. R. Illig and A. Prader, *J. clin. Endocr. Metab.* **30**, 615 (1970).
24. C. A. Birge, G. T. Peake, I. K. Mariz and W. H. Daughaday, *Endocrinology* **81**, 195 (1967).
25. L. Lumeng, W. F. Bosron and T. K. Li, *Biochem. Pharmac.* **28**, 1547 (1979).
26. R. K. Bonnichsen and N. G. Brink, in *Methods in enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 1, p. 495. Academic Press, New York (1955).
27. Y. Israel, L. Videla, V. Fernadez-Videla and J. Bernstein, *J. Pharmac. exp. Ther.* **192**, 565 (1975).
28. R. D. Hawkins, H. Kalant and J. M. Khanna, *Can. J. Physiol. Pharmac.* **44**, 241 (1956).
29. C. S. Lieber and L. M. DeCarli, *Science* **162**, 917 (1968).
30. F. Tobon and E. Mezey, *J. Lab. clin. Med.* **77**, 110 (1971).
31. L. Videla, J. Bernstein and Y. Israel, *biochem. J.* **134**, 507 (1973).
32. G. D. Wendell and R. G. Thurman, *Biochem. Pharmac.* **28**, 273 (1979).
33. G. L. S. Pawan, *Nature, Lond.* **220**, 374 (1968).
34. R. G. Thurman, W. R. McKenna and T. B. McCaffrey, *Molec. Pharmac.* **12**, 156 (1976).
35. A. I. Cederbaum, E. Dicker, C. S. Lieber and E. Rubin, *Alcoholism: Clin. expl Res.* **1**, 27 (1977).
36. E. R. Gordon, *Alcoholism: Clin. expl Res.* **1**, 21 (1977).
37. F. Moreno, R. Teschke and G. Strohmeyer, *Biochem. biophys. Res. Commun.* **89**, 806 (1979).
38. C. S. Lieber and L. M. DeCarli, *J. Pharmac. exp. Ther.* **181**, 279 (1972).
39. E. Mezey, *Biochem. Pharmac.* **21**, 137 (1972).