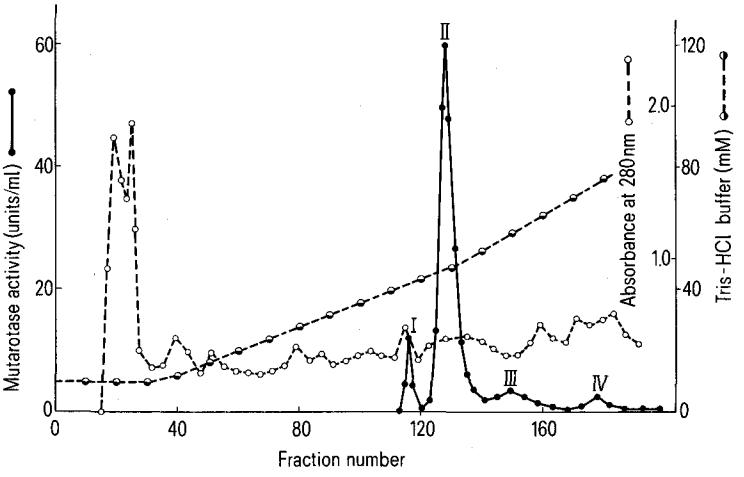

DEAE-cellulose column chromatography of human kidney mutarotase. Column bed, 2.5×30 cm; flow rate, 25 ml/h; eluting buffer, 1.2 l of linear Tris-HCl buffer gradient (10 to 120 mM, pH 7.2). Fractions of 5 ml were collected.

activity was at 7.4 with 0.02 M EDTA buffer. These properties of mutarotase type II were almost the same as those of the enzyme (type II) from rat kidney. However, no cross-reaction could be observed between purified human kidney mutarotase type II and antiserum raised in rabbit against rat kidney mutarotase type II.

Bailey et al.[4] have reported that about 50% of serum samples taken from patients with known renal disease elevated mutarotase levels and that the enzyme was absent from urine samples of all individuals studied. On the other hand, we have recently found that the kidney mutarotase appears in both serum and urine of patients[10] and rats[11] with nephrotic syndrome. We are now studying the cellular localization of mutarotase in rat kidney with the antibody against mutarotase type II from rat kidney, and which form of mutarotase appears in serum and urine of patients with nephrotic syndrome in order to obtain a clue for elucidating the physiological function of mutarotase.

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Effect of sucrose on lipogenesis of rats chronically treated with ethanol[1]

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Summary. The effect of chronic ethanol administration with and without sucrose on the lipogenic enzymes of liver and adipose tissue of rats was studied. Ethanol markedly influenced the adipose lipogenic enzymes at 28 days. Sucrose caused a 2–10fold increase in lipogenic enzymes of both adipose and liver.

Research into the effects of ethanol is tied to the use of isocaloric control experiments. In the rat model, the control experiment often involves animals given an isocaloric carbohydrate solution. DeCarli and Leiber[2] have proposed a method using a totally liquid diet to administer ethanol with carbohydrate used as an isocaloric control. This approach has the advantages of being convenient and allowing direct measurement of caloric intake plus the ethanol-treated animals gain weight at a rate comparable to liquid diet controls. It has been our experience, however, that animals dosed in this manner do not gain weight as rapidly as those allowed free access to rat chow, especially over long experimental periods[3]. In an attempt to find a method of ethanol dosing that would allow normal weight gain, experiments were performed in which some effects of sucrose were observed that are pertinent to ethanol research.

Materials and methods. Male Holtzman rats weighing 300–320 g at the start of the experiment were used. In 1 group, 15 animals were given ethanol daily (4 g/kg) as a 50/50 (v/v) solution in normal saline by stomach tube. 15 control animals received an equal volume of normal saline.

In the 2nd dosing procedure, 15 animals were given a mixture of 25% sucrose and 25% ethanol in water (w/v/v) as their only source of fluid, and an equal number of animals received a 25% solution of sucrose only. All animals were allowed free access to chow and were weighed on alternate days and the dosage adjusted accordingly.

At the end of 28 and 56 days, 6 animals from each of the 4 groups were sacrificed by decapitation and the livers and epididymal fat pads rapidly excised and chilled in ice-cold 1.15% KCl. The livers were homogenated in a glass-teflon homogenizer using 0.05 M potassium phosphate buffer, pH 7.4 containing 1.0 mM EDTA and 1.0 mM DTT. Adipose tissue was homogenized in a glass homogenizer using the same buffer. The particle-free supernatant solution was prepared by centrifuging the homogenates at 105,000 × g for 60 min.

Table 1. The effect of sucrose and ethanol plus sucrose on the activity of several lipogenic enzymes in rat liver and adipose tissue. Control animals received isocaloric amounts of sucrose only

Enzyme activity (nmole/min/mg protein)	28 Days treatment Sucrose control	Sucrose ETOH	56 Days treatment Sucrose control	Sucrose ETOH
	Adipose			
G6PDH	113.0± 9.0*	108.0± 2.10	165.0± 11.7	85.2± 9.9**
ME	85.0± 11.0	48.0± 10.0**	119.7± 12.0	31.1± 9.5**
FAS	45.0± 4.6	32.8± 4.6	43.7± 1.6	26.4± 5.6**
CCE	73.0± 9.0	53.0± 10.0		
	Liver			
G6PDH	57.5± 5.6	60.8± 7.7	69.7± 5.4	47.0± 3.5**
ME	32.5± 2.6	15.3± 2.9**	33.1± 3.4	15.5± 1.0**
FAS	18.2± 1.5	16.3± 1.6	17.1± 0.6	18.1± 0.9
CCE	55.2± 4.0	57.8± 1.5		

Abbreviations used: G6PDH, glucose-6-phosphate dehydrogenase; ME, malic enzyme; FAS, fatty acid synthetase; CCE, citrate cleavage enzyme. *Values represent mean±SEM for at least 6 animals. ** Differences are statistically significant ($p < 0.05$) relative to the control at the same time period.

Table 2. The effect of ethanol on the activity of several lipogenic enzymes in rat liver and adipose tissue. Ethanol (in saline) was administered by stomach tubing and control animals received only an equal volume of normal saline

Enzyme activity (nmole/min/mg protein)	28 Days treatment Control	ETOH tubed	56 Days treatment Control	ETOH tubed
	Adipose			
G6PDH	55.5± 2.0*	75.5± 2.5**	45.2± 2.3	49.7± 3.3
ME	10.6± 1.2	19.2± 4.8	8.0± 1.0	8.7± 1.5
FAS	9.1± 1.0	17.2± 1.7**	4.8± 0.2	6.3± 0.7
CCE	12.1± 0.9	23.1± 2.5**		
	Liver			
G6PDH	27.0± 2.4	28.1± 1.8	12.9± 0.7	15.2± 0.8
ME	19.2± 2.7	19.2± 2.3	12.9± 0.7	14.4± 0.8
FAS	7.4± 0.2	8.8± 0.5	3.4± 0.2	4.6± 0.2**
CCE	25.9± 2.8	29.3± 1.1		

Abbreviations as in table 1. *Values represent mean±SEM for at least 6 animals. ** Differences are statistically significant ($p < 0.05$) relative to the control at the same time period.

All enzymes were assayed spectrophotometrically and the assays were performed under conditions of saturating substrate with the amount of the enzyme as the rate-limiting component of the in vitro reaction. Protein concentration of liver and adipose preprations was determined by the biuret[4] and Lowry[5] methods, respectively.

Observed differences were tested for statistical significance by a one-way analysis of variance following calculation of mean and SEM values for each group. Significance at the $p < 0.05$ level was obtained using a two-tailed Student's t-test.

Results and discussion. Table 1 shows the influence of sucrose and ethanol on several lipogenic enzymes from liver and adipose. As has been reported previously[6], the enzyme activities were markedly increased by feeding the animals carbohydrate. Ethanol plus sucrose further influenced several of these enzyme activities. In these animals, sucrose plus ethanol actually caused significant depression of adipose and liver malic enzyme activity compared to sucrose treated. Similar results have been reported for this enzyme[7]. At 28 days, the ethanol tubed animals (table 2) had significant increases in all adipose enzymes measured except malic enzyme which showed a strong, similar trend.

After 56 days of treatment, the same relationship with respect to sucrose held, i.e. sucrose or sucrose plus ethanol elevated every enzyme in both tissues with ethanol suppressing the elevation somewhat. In this time period, only fatty acid synthetase from liver was elevated significantly by administration of ethanol by stomach tube (table 2). This latter result is contrary to the report of Savolainen et al.[8] where the fat/carbohydrate ratio was held constant for ethanol treated and control animals.

It has been pointed out by several investigators that sucrose is not without effect on liver[9,10] and that the response of lipogenic enzymes to ethanol is strain-related[11]. This work has further shown that depending on whether or not sucrose is used in the control, ethanol can increase or decrease the activities of these enzymes, particularly in rat adipose tissue. These results point out the difficulty of interpretation and possible errors of treating control animals with sucrose in experiments dealing with the effects of ethanol on lipid metabolism.

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