Effect of diabetes on the vertebral column of rats and its modification by estradiol[1]

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Summary. In vertebrae of genetically selected sucrose-fed diabetic rats a statistically significant bone deficit was found after diabetes had been present for about 8 months. No osteopenia was observed in diabetic rats following treatment with estrogenic hormone for 5–7 months. The development of osseous centers in the end plates of the vertebrae was retarded in diabetic rats, but was about normal in diabetic rats given estrogen. – No differences were noted in the growth zones or in the tendency to develop articular lesions in rats of the various groups. Possible differences in the amount of GAG in intervertebral discs of diabetic and non-diabetic rats respectively await further confirmation.

2 types of major skeletal changes are attributed to the metabolic state in diabetes – as opposed to local lesions secondary to infections, angiopathy and neuropathy – firstly, a generalized bone deficit, osteoporosis and secondly, hyperostotic spondylosis, a disorder of the spine associated with osteophytic outgrowths at the vertebrae[3-9]. The mechanisms by which these lesions arise are still under discussion, and experimental evidence obtained in animals with spontaneous or induced diabetes is still scanty. Therefore, a number of investigations using diabetes-prone laboratory animals are being carried out at present[10]. The following report deals with the response of the vertebral column of rats made diabetic by diet alone[11] and with the modification of this response by estradiol (E).

Material and methods. Genetically selected sucrose-fed diabetic rats were studied. These animals are characterized by their propensity to develop diabetes when fed a high sucrose diet[11]. Rats not possessing this trait served as controls. 16 animals from the time of weaning on were fed the diabetogenic diet containing 18% casein, 5 butter, 72% sucrose, and 5% vitamins and salts. 8 diabetic rats were given s.c. injections of 0.25 mg estradiol benzoate in 0.1 ml olive oil once a week for 5–7 months from the age of 2 months on and 8 diabetic rats received 0.1 ml olive oil only. 8 non-diabetic rats also received 0.1 ml of oil s.c. B.wt, oral glucose tolerance, spontaneous blood glucose and plasma lipids were determined as in previous studies. The animals were killed at 10 months of age or somewhat earlier, if they had developed massive albuminuria. At autopsy, kidneys and knee joints were removed for separate examination. For the purpose of the present study, the vertebral columns were dissected, fixed in 4% formaldehyde, decalcified in a mixture of equal parts of 45% formic acid and 20% sodium citrate in water, split sagitally, washed in running water for 24 h, dehydrated in ethanol, cleared in methylsalicylate and embedded in celloidin-paraffin. Sections stained with hematoxylin and eosin and with alcian blue and PAS or nuclear fast red, were evaluated for findings at the growth zones, the terminal plates and the small joints of the vertebrae and in the intervertebral discs. The metaphyseal spongiosa was quantitatively assessed in 29 vertebrae of 8 diabetic rats, in 46 vertebrae of 8 normal rats, and in 28 vertebrae of 8 diabetic rats given estrogen, using an electronic point counting apparatus connected to a computer (Contron Co. Model MOP-AMO 3)[2]. The area assessed included the entire metaphysis from cortex to cortex and from the subchondral osseous lamella to the distal end of the metaphysis, which was characterized by a small bony prominence extending inward from the endosteum.

Results and discussion. The results are tabulated in the table. The vertebral growth zones of rats of the 3 groups were equally inactive, but the groups differed as to the amount of cancellous metaphyseal bone present. In the diabetic rats, spongiosa occupied 12.5–32% of the entire metaphysis (median 20.5%) as compared to 14.0–43% (median 25.3%) in non-diabetic rats and to 12.7–43.3% (median 24.9%) in diabetic rats given E ($p=0.01$). The variations were due to variations in several vertebrae of the same rat as well as to variations between individual animals.

Consistent with the bone deficit in the diabetics was the retarded development of the osseous centers in the end plates indicating decreased osteogenesis in the diabetic rats. In accordance with earlier clinical and experimental data[3,5-9], there was no evidence of increased resorption of bone in diabetic animals. The absence of the bone deficit after treatment with estrogen may, however, be due to an inhibition of bone resorption by the hormone, such as occurs also in non-diabetic rats[13].

Evidence of spondylosis and spondylarthrosis was negligible in all groups of rats, either because the animals had not reached a critical age or because they might be genetically resistant to the development of the lesions.

There was some decreased staining with alcian blue in the articular cartilage and intervertebral discs of diabetic rats as compared to rats of the other groups, indicating a decrease in the amount of glycosaminoglycans (GAG) present. If further studies using methods for more accurate determination of GAG in tissues[14,15] confirm these findings, they

Table showing biochemical and morphometric findings in control and experimental rats

	Non-diabetic rats	Diabetic rats	Diabetic rats, estradiol-treated
Number of animals	8	8	8
Weight (g)	477 ± 83.0	278 ± 29.0*	194 ± 9.1**
Spontaneous blood glucose (mg%)	111 ± 4.5	205 ± 10.2*	210 ± 17.0*
Glucose tolerance (mg%)	413 ± 15.0	979 ± 79.0*	1102 ± 71.0*
$\sum$ G 0 + 30 min + 60 min + 120 min			
Cholesterol (mg%)	65 ± 5.4	134 ± 36.0	196 ± 22.3*
Triglycerides (mg%)	57 ± 10.8	253 ± 86.0*	102 ± 13.6*
Plasma estradiol (pg)	–	146 ± 0.5	308 ± 28.0
Difference glomerulosclerosis	–	43% (3/7)	60% (5/8)
Metaphyseal spongiosa, vertebrae (%)	25.5	20.5*	24.9**

* Significance vs non-diabetic rats, $p<0.05$. ** Significance vs diabetic, not treated with estradiol, $p<0.05$.

would be in agreement with the decrease of dermal and aortic GAG observed in diabetic rats[16,17]. The absence of this effect after treatment of the diabetic rats with E is of interest in view of observations suggesting that the hormone does not only inhibit the synthesis[18,19], but also decreases the degradation of GAG[20].

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Effect of acute and chronic ethanol ingestion on the content of reduced glutathione of various tissues of the rat[1]

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Summary. The effect of acute ethanol ingestion (5 g/kg) by fasted rats, or chronic treatment in fed animals, revealed a significant decrease in the content of reduced glutathione of the liver and kidney. No changes were observed in reduced glutathione levels of the pancreas, intestines, stomach or spleen in the acute model. In this condition, the time course study of the decrease in reduced glutathione levels showed a progressive effect in the liver and a rapid and constant effect in the kidney.

Hepatic reduced glutathione (GSH) seems to play a significant role in the protective mechanisms against the toxic effects of some xenobiotics and/or their metabolites[2]. It has been suggested that GSH depletion could lead to lipoperoxidation and, consequently, to cell damage and lysis[3]. Previous studies by our group have shown that the administration of a single dose of 5 g of ethanol/kg to rats fasted overnight induced a drastic decrease in the content of liver GSH after 6 h of intoxication[4], concomitant with an enhancement of lipoperoxidative processes[4,5]. Since the metabolic and toxic effects of ethanol consumption are not limited to the liver, but occur in several other organs as well[6], it was of interest to study the effect of acute ethanol administration to rats on the content of GSH of tissues such as kidney, pancreas, intestines (jejunum), stomach and spleen, including the liver for comparison. GSH levels in liver and kidney were studied in animals following chronic ethanol ingestion, a situation in which hepatic lipoperoxidation has been found to increase markedly[7–9].

Materials and methods. Studies of the effect of acute ethanol ingestion were carried out in male Wistar rats (Facultad de Medicina Occidente, Universidad de Chile) weighing 150–200 g and fasted overnight (16 h), intubated with 5 g of ethanol/kg as a 40% w/v solution in saline. Control animals received isovolumetric amounts of saline or isocaloric amounts of glucose[4]. Determinations were done after 6 h of treatment in animals kept in a warm environment (25 °C). The time course study of changes in liver and kidney GSH levels was performed in fasted rats given 5 g of ethanol/kg sacrificed after 1, 2, 3, 4, 5 and 6 h of intoxication.

In studies involving chronic ethanol administration, male Wistar rats (Canadian Breeding Laboratories, Quebec) weighing 168.2 ± 1.0 g ($n = 12$) were matched by weight. Half of the animals were given an ethanol-containing liquid diet (composition as percent of total calories: 35% ethanol, 19% protein, 41% fat and 5% carbohydrate) and the other half received a liquid diet in which ethanol was replaced isocalorically by sucrose[10], for 4 weeks. The caloric intake was 310 ± 4 kcal/kg/day and the ethanol consumption was 15.5 ± 0.2 g/kg/day ($n = 21$ determinations in 6 pairs of rats). In order to minimize possible differences in the time of food intake preceding the experiments, which could alter GSH levels[4], 1/3 of the diet consumed by the ethanol-treated rat the day before was given at 14.00 h to the corresponding pair of animals and the remaining 2/3 at 21.00 h. On the following day, 09.00 h, the animals were given 4 ml/100 g b.wt of the respective liquid diets by gastric tube[11], 2 h before sacrifice.

For measurements of the GSH content, the tissues were washed in cold 0.15 M KCl, homogenized in 0.5 N $HClO_4$ and centrifuged at 4500 rpm for 10 min. GSH was immediately determined in aliquots of the supernatants obtained with 5,5′-dithiobis-(2-nitrobenzoate) at 412 nm according to Ball[12]. All reagents were obtained from Sigma (St. Louis). Results are expressed as means ± SEM and the statistical analysis was performed by Student's t-test.

Results. The administration of a single dose of 5 g of ethanol/kg elicited a 50% decrease in the concentration of GSH in the liver of fasted animals as compared to the corresponding control rats (table, A). Apart from the liver, the kidney also showed a significant decrease in GSH levels