compared to controls. These results are in agreement with those reported by Younoszai and Schedl[5], who found a similar increase in the disaccharidase levels on 5th day of alloxan administration. Our results with chronic alloxan diabetic animals obviously suggest that increase in the sucrase and lactase activities may not be due to toxic effects of alloxan, which could be prevalent in short-term experiments. Furthermore, the enhanced activity of these enzymes cannot be attributed to increase in intestinal cell mass or its surface area observed in diabetes[15], since there is no change in the activity of brush border AP (table 2), which is known to be located on the same site of mucosal membrane as the disaccharidases[16]. Insulin administration to diabetic animals restored the activity of these enzymes to almost control levels. The specific increase in the level of these enzymes is most likely due to elevated levels of glucagon, cortisone or catacholamines observed in diabetes[17,18], and is not due to the lack of insulin in this derangement, since insulin administration alone to control animals also augments the activity of sucrase and lactase. Increase in the concentration of these insulin antagonists also occurs in insulin induced hypoglycemia[19], which may again be responsible for the increased enzyme activities in insulin-treated animals. Recently Clenano et al.[20] showed that cortisone or tri-iodothyronine injection to pregnant female rats can elicit precocious appearance of jejunal sucrase in their foetuses.

There is no change in the activities of AP and Mg-ATPase in diabetic rats compared to controls, as revealed by the results shown in table 2; however, insulin treatment of the animals led to a marked increase of AP and an appreciable stimulation of Mg-ATPase activities. Increase in the uptake of sugars and amino acids in the intestine of insulin-treated animals has also been demonstrated[21,22]. Such a facilitative action of insulin on the enzyme systems could be due to the general anabolic action of this hormone on protein biosynthesis[23]. In view of the close functional link between sugar absorption process and the disaccharidases in intestine[24,25], it would appear that increased sugar uptake and the disaccharidase activities in diabetes and in hyperinsulinism is due to similar or identical mechanism(s).

In order to elucidate whether increase in the activity of disaccharidases in diabetes is the result of a new enzyme formation with high substrate affinity or increased maximum velocity, we studied the kinetics of sucrase in control and in diabetic animals. Kinetic parameters calculated from the double reciprocal plot (figures not shown) indicate that there is no change in K_m of the enzyme in diabetic and control animals ($K_m = 24.4$ mM and 26.3 mM in diabetic and control groups respectively). But V_{max} (μmoles glucose/min mg protein) increases from 1.79 in control to 3.33 in diabetic animals. This clearly suggests a net increase in the enzyme content.

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Sex difference in polyethylenglycol-induced thirst

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Summary. The polyethylenglycol-induced thirst in male and female castrated rats has been studied. The polyethylenglycol (PG) increases the water intake more in females than in males. Estradiol benzoate and testosterone P. diminishes the amount of water drunk after PG treatment in the females, but not in the males.

Extracellular thirst can be stimulated by reducing the volume of extracellular fluid, without changing the general osmolarity or the volume of the intracellular compartment. This can be done by injecting i.p. or s.c. a hyperoncotic colloidal solution of polyethylenglycol (PG)[1,2]. It was proved that the injection of PG produces an acute edema in the area of the injection and an increase in the activity of the plasmatic renin (PRA)[2]. This increases of the PRA provokes the formation of greater quantities of angiotensin-II, which, as is know, is a powerful dipsogen when administered in different ways[2-5].

Because the food intake seems to be related to sexual factors[6-8], this would be possible also for water intake. We have seen[9] that the administration of angiotensin-II produces different effects on the water intake in male and female rats. The females ingest more water than the males when stimulated with angiotensin-II. A progressive pattern of differentiation was observed between the 2 sexes when the animals were castrated at different levels of their development. The males and females castrated at birth drunk exactly the same volumes of water when, as adults, they were injected with angiotensin-II. The differences started when the animals were castrated before puberty or as adults. The object of the present study is to clarify the real significance of sex, and the role of the sexual hormones in the extracellular thirst.

Materials and methods. Rats of both sexes of the Wistar breed were used. When weighing 150 g, they were castrated and kept in boxes of plastic and wire (22×35×22 cm) in groups of 4 animals, separated by sex. A cycle of 12 h light and 12 h dark, and a temperature of 20 °C with small fluctuations, were maintained. Throughout the whole time, the animals were given water and standard rat food (Sanders) ad libitum. After more than a month after castration, the experiments were commenced.

Experimental groups. 1. A group of 10 castrated males and 10 castrated females was selected and put in individual plastic boxes. With food and water ad libitum, at midday of the 5th day each animal was weighed and the standard drinker was substituted by a graduate tube of 20 ml and accuracy of 0.1 ml. After 6 h, the volume ingested by each animal was measured.

2. With procedure and animals similar to those in the previous group. On the 5th day, they were injected s.c. with saline (ClNa 0.9%, 15 ml/kg b.wt), and water intake was measured in the next 6 h.

3. This group received, on the 5th day of individual caging, 1 s.c. injection of PG (Carbovax, 1540, Doesder) 15 ml/kg b.wt, PG 30% in saline.

4. The animals of this group were injected i.m. from the 3rd day of individual housing with an oleagenous solution of propionate of testosterone (3 mg/kg b.wt). On the 5th day, the water ingestion during a period of 6 h was measured. The following day these animals received, as well as testosterone P., 1 injection of PG, the same as the previous group, and their water intake was measured.

5. A group the same as the previous one, but the testosterone P. was substituted by the same dose of estradiol benzoate.

Results. When comparing the intake of males and females using the Student t-test, we find that there are significant differences only in the group treated exclusively with PG ($t = 5.02$; d.f. = 17.00; $p < 0.001$).

Water intake of male and female castrated rats

	Males $\bar{X} \pm$ SE (n)	Females $\bar{X} \pm$ SE (n)
Nontreated	11.08 ± 2.18 (10)	8.20 ± 2.15 (10)
Saline	2.68 ± 1.32 (10)	5.14 ± 1.95 (10)
PG*	17.42 ± 2.41 (10)	38.77 ± 3.62 (9)
Testosterone	6.15 ± 1.62 (9)	3.06 ± 0.67 (7)
Estradiol	4.71 ± 1.19 (10)	4.21 ± 0.90 (14)
Testosterone + PG	17.96 ± 1.77 (9)	22.36 ± 2.90 (9)
Estradiol + PG	18.04 ± 2.14 (10)	13.58 ± 1.21 (8)

$\bar{X}$ = Mean values of water intake in 6 h, in ml/kg b. wt. (n) = Number of rats. * $p < 0.001$. Comparison between males and females (Student t-test).

In the males, the minimum corresponds to those injected with saline, followed by those treated with estradiol benzoate and testosterone P. The maximum was shown in those injected with PG. The analysis of the variance reveals the existence of significant differences between the different values ($f = 17.70$; d.f. = 6.61; $p < 0.001$).

The analysis of the variance of data of intake of the females reveals also the existence of statistically significative differences ($f = 32.73$; d.f. = 6.60; $p < 0.01$). The minimum intake corresponds to the females treated with testosterone P., followed by the group treated with estradiol. The maximum was shown in those injected with PG.

Discussion. The study of sexual differences of water intake and thirst is an almost unexplored field. Often, the lack of interest in a particular subject is due to the lack of outstanding facts easily observed. That is why it is not strange that in the 1st group that we studied, castrated control animals without treatment, differences between the 2 sexes have not been observed. Nevertheless, the levels of intake of castrated males and females evolved in a different way when treatment was applied. Whilst the saline injection did not change significantly the ingestion of the females, it reduced that of the males ($p < 0.01$). From these results it can be deduced that there exists a sexual difference related to the adjustment of the mechanism of the regulation of the extracellular thirst after the injection of saline solution.

The dependent sexual differences are shown in a striking manner in the castrated animals when s.c. injected with PG, which diminishes their volemia. Although there is a significant increase in the ingestion of water in the males as well as in the females, in the latter the volume drunk is more than double that of the males. From this it can be deduced that there is an exaggerated sensitivity in the castrated females to extracellular thirst. The observation that the stimula of a negative character, like the saline injection, with the subsequent increase of extracellular fluid volume, do not produce a statistically significative decrease in the intake of the females, together with the observation that the positive stimulus (injection of PG) provokes an enormous reaction, seems to prove the existence of a set-point in the mechanism of regulation of extracellular thirst different in castrated females and males.

The administration of hormones does not change the reaction of the males when they are treated with PG. This invariability of water intake in the males stimulated with PG and treated with testosterone P. or estradiol benzoate, compared with the marked decrease in the PG-induced thirst in the females when injected with these hormones, is new evidence of the existence of a difference between the sexes in the control of thirst.

We still do not know at which level the differentiation that we have detected is carried out, this needs further study. Now that the mechanisms of sexual differentiation of diverse nervous structures are well known[12-15], we believe that it would be of great interest in the future to study their possible relations with some aspects of regulation of ingestive behaviour and hydric balance.

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