Effect of caerulein infusions on serum calcium and phosphorus levels

Caerulein infusions	Time (min)							Nadir value
	0	15	30	45	60	90	120	
Calcium (60 min)	10.1 ± 0.24	9.7 ± 0.24[c]	9.4 ± 0.27[c]	9.2 ± 0.27[c]	9.3 ± 0.27[c]	9.4 ± 0.22[c]	9.5 ± 0.22[b]	9.0 ± 0.24[c]
Phosphorus (60 min)	3.0 ± 0.14	2.8 ± 0.18	2.8 ± 0.19	2.7 ± 0.21	2.7 ± 0.19	2.6 ± 0.20[b]	2.8 ± 0.24	2.3 ± 0.15[b]
Calcium (15 min)	9.8 ± 0.50	8.3 ± 0.38[c]	8.2 ± 0.26[c]	7.9 ± 0.20[c]	7.7 ± 0.45[b]	7.8 ± 0.26[b]	8.3 ± 0.33[a]	7.6 ± 0.28[c]

Means ± S.E.M. are indicated. [a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.005$ vs the 0 min value.

^{47}Ca injected i.v.[4,5], we have also evaluated the effect of caerulein infusion on the disappearance rate of ^{47}Ca.

Materials and methods. The experimental program consisted of two studies and was carried out on a total of 24 normal subjects, aged 59–85 years (mean 71.6). In the first study, 8 subjects underwent an i.v. caerulein infusion (4 ng/kg body wt./min for 60 min) and 7 subjects underwent a similar caerulein infusion (4 ng/kg body wt./min for 15 min). Blood samples were taken on both occasions at 0, 15, 30, 45, 60, 90 and 120 min and tested for serum Ca levels[6]. In the first 8 subjects, serum phosphorus levels were also determined[7]. In the second study, the effect of caerulein on radioactive Ca kinetics was investigated in 2 groups of subjects (4 subjects for each group) by infusing, respectively 60 min and 90 min after the injection of 50 mCi $^{47}CaCl_2$, caerulein at a dosage of 4 ng/kg body wt./min for 15 min. Venous blood samples were taken at 5, 20, 60, 65, 75, 85, 100 and 120 min in the first group and at 5, 45, 90, 95, 100, 105, 115, 130, 150 and 180 minutes in the second group, and evaluated for specific radioactivity by conventional scintillation counting techniques.

The tests were always begun at about 09.00 h after an overnight fast and at least 1 h of bed rest.

Results. The Table indicates the effect of the 2 caerulein infusions on serum Ca and P levels; the reduction of serum Ca levels was statistically significant, either after the 60 or the 15 min infusion, in every point of the curves. Following the first caerulein infusion, a slight decrease of serum P levels was also observed, significancy being however reached only at the 90 min point. The nadir value of serum Ca was reached at approximately 72 min and at 73 min respectively after the first and the second caerulein infusion; the nadir value of serum P levels was reached at the same time.

In the second study, caerulein infused 60 or 90 min after 50 mCi $^{47}CaCl_2$, increased the specific plasma radioactivity. Owing to the few data available, no statistical analysis was performed.

Discussion. In the present study we have shown that the administration of caerulein, a natural polypeptide similar in its chemical structure to the C-terminal octapeptide of CCK pancreozymin, is able to induce hypocalcemia in normal subjects. The degree of hypocalcemia detected in our patients is similar to that induced by calcitonin. This result, taken together with the findings of CARE et al.[2] of a stimulatory role of caerulein on calcitonin secretion from isolated guinea-pig thyroid in vitro, suggests that caerulein elicits calcitonin release also in man.

In addition, caerulein has been shown to affect the regression curve of ^{47}Ca, in a manner similar to calcitonin, though with a little delay. This delay would indicate that caerulein does not act directly on calcium levels, but via a stimulation of calcitonin secretion.

Taken together, our results would indicate that caerulein stimulates in humans, as well as in the guinea-pig, calcitonin secretion. However, at present we cannot rule out the possibility that caerulein affects directly calcium metabolism, mimicking calcitonin effects.

In any case, the effect of caerulein on calcium metabolism is of great interest, in that, if our results will be confirmed, caerulein would be of advantage in those bone diseases in which calcitonin has proved helpful[8].

Summary. Caerulein, infused in normal subjects, significantly reduces serum Calcium levels; in addition, when infused 60 or 90 min after radioactive calcium, it increases the specific plasma radioactivity, in a manner similar to calcitonin. These results suggest that in man caerulein stimulates calcitonin release.

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Chronic Treatment of Immature Male Rats with Synthetic LH-RH[1]

The neuroendocrine mechanisms which initiate the sexual maturation are not well known. It seems that the production and liberation of gonadotropin-releasing hormones by the hypothalamus, the secretion of gonadotropins by the pituitary and the production of sex hormones by the gonads are determinant factors[2]. Studies carried out in maturing animals[3] and prepubertal

[1] LH-RH (Synthetic LH-releasing hormone), was kindly supplied by Serono Laboratories, Rome, Italy.

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Immature rats chronically treated with LH-RH. Differential cell counts

Age (days)		1	2	3	4	5	6	7	8
Controls	Sup. Cell	12.80 ± 0.46[a]	16.04 ± 0.35	16.52 ± 0.43	23.56 ± 0.58	27.88 ± 0.61	25.92 ± 0.74	31.24 ± 0.89	40.24 ± 1.01
	Go	2.00 ± 0.14	1.56 ± 0.13	1.72 ± 0.19	1.32 ± 0.20	0.88 ± 0.16	0.64 ± 0.15	0.52 ± 0.14	0.52 ± 0.13
	Cy	—	—	—	—	—	—	—	—
LH-RH treated	Sup. Cell	14.00 ± 0.46	15.08 ± 0.49	16.24 ± 0.43	22.56 ± 0.53	29.04 ± 0.88	27.52 ± 0.97	31.12 ± 0.87	40.48 ± 0.95
	Go	2.44 ± 0.19	1.78 ± 0.12	1.96 ± 0.20	1.20 ± 0.17	0.88 ± 0.16	0.72 ± 0.15	0.52 ± 0.14	0.52 ± 0.12
	Cy	—	—	—	—	—	—	—	—

Age (days)		9	10	11	12	13	14	15
Controls	Sup. Cell	36.12 ± 0.78	35.44 ± 0.78	35.16 ± 0.92	36.12 ± 0.55	35.32 ± 0.87	35.72 ± 0.59	35.00 ± 0.55
	Go	0.52 ± 0.13	1.16 ± 0.09	3.48 ± 0.26	2.40 ± 0.22	5.36 ± 0.23	9.92 ± 0.52	12.04 ± 0.52
	Cy	—	—	—	—	2.88 ± 0.36	6.36 ± 0.85	11.96 ± 1.25
LH-RH treated	Sup. Cell	35.04 ± 0.88	35.00 ± 0.85	34.64 ± 0.73	36.24 ± 0.75	34.36 ± 0.81	35.40 ± 0.67	34.72 ± 0.65
	Go	0.48 ± 0.13	1.20 ± 0.10	2.72 ± 0.20	2.28 ± 0.20	5.08 ± 0.28	6.88 ± 0.42	12.04 ± 0.49
	Cy	—	—	—	—	2.92 ± 0.36	5.80 ± 0.68	11.56 ± 0.84

[a] All values are mean number of cells per tubular cross section ± SEM. Sup. Cell, supporting cells and/or Sertoli cells; Go, gonocytes, primitive type spermatogonia and/or spermatagonia; Cy, primary spermatocytes.

boys[4,5] showed that the pituitary is able to respond to a single injection of LH-releasing hormone (LH-RH) with an increase of LH and FSH plasma levels.

This work was carried out in order to explore the possibility that LH-RH might act as a trigger of the sexual maturation process. We intend to investigate whether this preparation, chronically and precociously administered to immature rats, might be able to accelerate the initiation of spermatogenesis and the final differentiation of Leydig cells.

Material and methods. Two similar groups of male Wistar rats were used, one of them as control. The animals of the second group were daily injected s.c. with the synthetic LH-RH from 1st to the 15th day of age; 5 μg of LH-RH were given to each rat at 08.00, 12.00, 16.00 and 20.00 h. The daily dose of 20 μg was given fractionated, due to the proved short half-life of this preparation after injection[6]. LH-RH was dissolved in 0.1 ml of a solution containing 8% of gelatin, 0.9% sodium chloride and 0.057% of acetic acid. The control animal received 0.1 ml of the same solvent 4 times a day.

After beginning the treatment, some animals from both groups were killed every 24 h, 2 h after the last injection. Testes were removed and fixed in Bouin's fluid. Paraffin sections were stained with hemalumeosin.

The chronology of appearance of mature Leydig cells and the development of the spermatogenesis was determined. The germinal epithelium was evaluated by means of differential cell counts. To this end, supporting (or Sertoli cells), spermatogonia (primitive or adult types) and primary spermatocytes were counted in 25 transversal sections of seminiferous tubules and the mean per tubules was determined. These counts were statistically analysed by the *t*-test.

Results. Our results are summarized in the Table. It can be observed that, in the normal untreated animals, supporting cells (immature Sertoli cells) steadily increased in number until the 8th day after birth and remained unchanged afterwards. Then changes in the qualitative conditions began; many of these cells showed increasingly mature characteristics; around the 12th day, their maturation was almost complete.

Gonocytes appeared at birth at an average of 2.0 per tubular cross-section. Although they divide giving rise to intermediate and primitive type A spermatogonia[7], the average number of gonial cells decreased because many of these cells degenerated by the 9th day. Type A spermatogonia appeared and the average number of gonial cells per cross tubular sections progressively increased. From the 13th day on, other types of spermatogonias and spermatocytes are present and a sudden increase of the tubular diameter takes place. Mature Leydig cells appeared in the 10th day and noticeably increased after 13th day of age (Figures 1–6).

The sequence of events in the animals treated with LH-RH, as the Table shows, was not different from qualitative point of view respect of the control animals. Adult gonial types of germ cells appeared also around the 9th day and spermatocytes at the 13th day. Mature Leydig cells were not seen before the 10th day. Statistical analysis neither showed significant changes in the differential cell counts, except for day 14 which demostrated a higher number of spermatogonias in the untreated animals.

Discussion. It was observed in prepubertal boys with one cryptorchid testis that, after receiving daily 500 to 1000 μg of LH-RH for 1 month, the remaining scrotal testis showed no significant changes comparing with the testes of a normal population of the same age[8]. On the other hand, comparing before and after LH-RH treatment testicular biopsies of patients with prepubertal hypogonadotropic hypogonadism, the same authors

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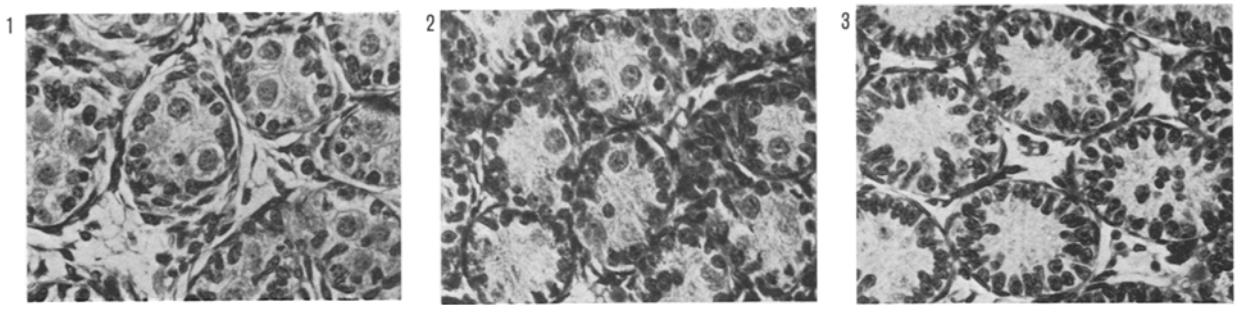

Fig. 1. Control 1-day-old rat testis. The seminiferous tubules show gonocytes and supporting cells. Occasional Leydig cells. HE, × 320.
Fig. 2. LH-RH treated, 1-day-old rat testis. Similar cell types. HE, × 320.
Fig. 3. Control 11-day-old rat testis. The tubular diameter and the number of supporting cells has increased. Primitive type A spermatogonia are seen. He, × 320.

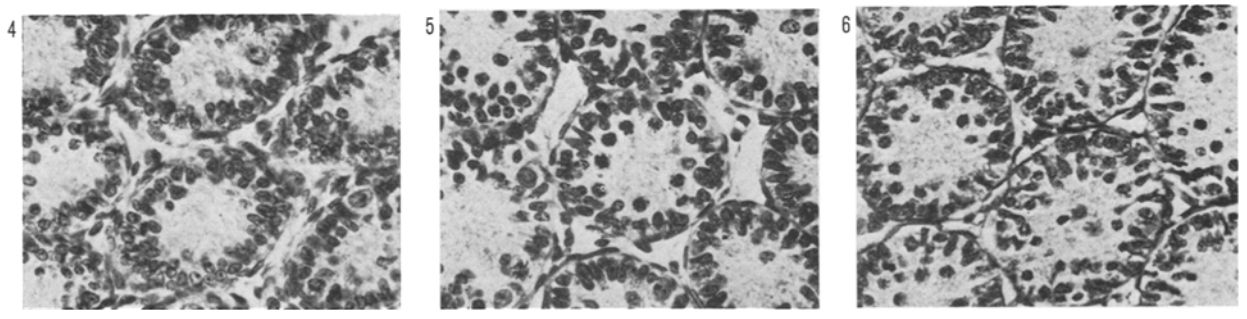

Fig. 4. LH-RH treated 11-day-old rat testis. Similar to the Figure 3. HE, × 320.
Fig. 5. Control 13-day-old rat testis. Tubular diameter increased. Adult type spermatogonia and primary spermatocytes are present in the germinal epithelium. HE, × 320.
Fig. 6. LH-RH treated 13-day-old-rat. Testes similar to the control. HE, × 320.

reported only slight stimulation of the testis. RAMIREZ and MCCANN[9] have suggested that the onset of 'puberty' in the rat is determined by a change in the hypophysis sensitivity to releasing hormones rather than by changes of the gonadotropin content of the glands. In human, ROTH et al.[4] stated that pubertal and adult testes appears to be more responsive than the immature testis in releasing testosterone after the administration of LH-RH. Also SANDOW and BABEJ[10] speculated that chronic LH-RH treatment may have a negative feed-back effect on pituitary sensitivity to the gonadotropin releasing hormones.

Our results showed that administered synthetic LH-RH is neither able to initiate per se the development of spermatogenesis and maturation of Leydig cells, nor to accelerate the normal rhythm of the natural evolution of the gonads. It is quite possible that other mechanisms might be involved in the onset of puberty. On the other hand, we had no evidence of a negative feed-back induced by the injected LH-RH, since both treated and untreated animals showed neither qualitative nor quantitative histological differences in the testis. The significance of the higher number of spermatogonial cells in untreated 14-day-old rats is open to question.

Resumen. Ratas machos inmaduros tratados crónicamente con factor liberador sintético de LH desde el primero hasta el 15 dia de edad a las dósis de 5 μg subcutáneos cuatro veces al dia cada cuatro horas por animal, fueron incapaces de iniciar o acelerar el proceso de maduración testicular estudiando el proceso espermatogénico por contages celulares diferenciales en secciones transversales y el desarrollo de las células de Leydig maduras.

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Effect of Castration on the Rat Pineal Gland: a Fluorescence Histochemical and Biochemical Study

Earlier investigations[1-3] demonstrated that some pinealocytes of the rabbit, rat, hedgehog and mole contain a yellow autofluorescent substance. In rabbit and rat pinealocytes, this material appeared to be a tryptophan-rich protein[1,2] the function of which is still unknown. It might possibly be a carrier protein of pineal hormones[2]. Considering this hypothesis, it seemed of interest to determine, in the rat pineal gland, the quantity of cells containing yellow autofluorescent material under varying

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