Difference in surface area of the corona cells of ova after incubation for 6 h in uterine secretion from rats pretreated with oestradiol, progesterone or testosterone

Substances	Doses s.c.	No. of assays No. of rats	Initial surface area (mm^2)	Surface area after 6 h incubation (mm^2)	Absolute increase (mm^2)
Controls	Vehicle only	12/36	1841 ± 108	4708 ± 348	2867 ± 377
Oestradiol	3 µg/kg	2/4	1191 ± 073	3509 ± 490	2318 ± 344
	10 µg/kg	2/6	1233 ± 212	3202 ± 1200	1968 ± 988
	30 µg/kg	2/6	1394 ± 048	3989 ± 076	2495 ± 159
Progesterone	3 mg/kg	2/6	2319 ± 020	4665 ± 561	2346 ± 582
	10 mg/kg	7/19	1401 ± 179	1664 ± 306	264 ± 209
	30 mg/kg	4/10	1254 ± 323	1145 ± 262	− 109 ± 082
Testosterone	3 mg/kg	3/8	1771 ± 056	4071 ± 240	2300 ± 184
	10 mg/kg	3/8	1697 ± 155	4823 ± 780	3125 ± 830
	30 mg/kg	3/8	1437 ± 376	1861 ± 910	424 ± 560

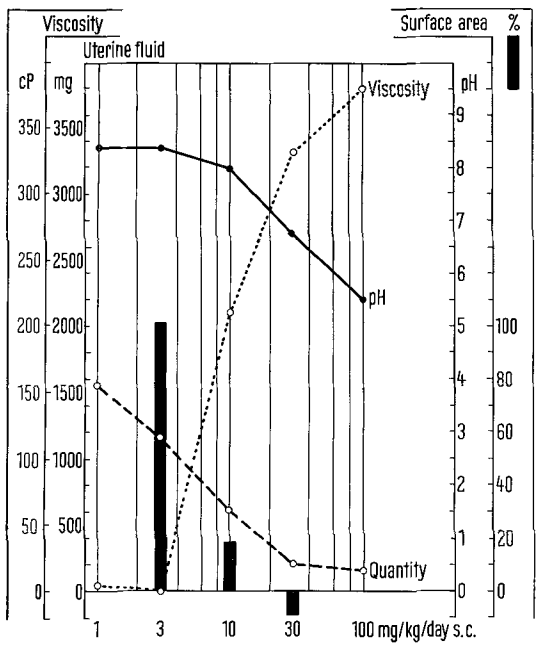

Fig. 3. Physical properties of uterine secretion from progesterone pretreated rats and action in vitro on dispersion of corona cells of the ovum.

PINCUS[6] and MCCLEAN et al.[7] assumed that this factor might be an enzyme similar to spermatozoal hyaluronidase. We have observed that the dispersion of the corona cells can be accelerated in vitro following addition of rat spermatozoa, which suggests that the factor responsible for this effect might well be of an enzymatic nature and that it can be inhibited by progesterone treatment.

Résumé. La ligature des extrémités distales des cornes utérines de rates castrées et sensibilisées à l'œstradiol (3 µg/kg/jour, s.c.) permet de recueillir au bout de 21 jours la sécrétion utérine accumulée. Celle-ci renferme un facteur provoquant in vitro la dispersion des cellules de la couronne de l'œuf. Cette action est inhibée par un prétraitement de progestérone (10 mg/kg/jour, s.c.) du 14è au 21è jour ou par de fortes doses de testostérone. L'œstradiol, par contre, ne présente pas cette action inhibitrice.

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Thyrocalcitonin and Citric Acid

The relationship of calcium and citric acid has been known for a long time[1]. A calcium-citrate complex in blood represents the non-ionized ultrafiltrable fraction of calcium and the amount of citric acid in serum may influence the quantity of ionized calcium. A whole number of hormones and other factors which induce changes of the serum calcium level can influence the citric acid too: parathormone, vitamin D, thyroxin, etc. Thyrocalcitonin (TC) reduces the serum calcium very quickly, and vice versa the production of the hormone is controlled by the actual quantity of calcium in the circulation. So far nothing is known about a possible action of TC concerning organic acids in general and citric acid in particular.

In our experiments we tried to establish whether the level of citric acid follows the changes of the serum calcium and whether it depends on time and dosage. TC was gained from the thyroid glands of pigs according to HIRSCH[2]. The purified extract in acetate buffer (pH 3.8) was injected i.m. to intact 50-day-old male rats of the Wistar strain (100–120 g) which were kept for 4 days on a calcium-free diet. All experimental and control animals

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were killed at given intervals, blood samples were collected by heart puncture for an analysis of calcium[3] and citric acid[4].

The results, given in Tables I and II, show that after the administration of TC the decrease of serum calcium and citric acid is simultaneous. Similar behaviour of these 2 components is well known in cases of hypoparathyroidism. In hyperparathyroidism the rise of the citric acid and calcium concentrations in serum is connected with the destruction of bone tissue on one side and the influence of parathormone on the kidneys on the other side. The effect of TC on bone tissue is antagonistic to that of parathormone. It inhibits the reabsorption of bone mineral[5,6], enhances the incorporation of calcium into the bones[7], increases the number of osteoblasts[8] and thus favours the formation of new bone. In spite of these findings, the fast decrease of serum calcium and citric acid cannot be explained only by the effect on bones mentioned above, because of the time factor. Two possible mechanisms of action may be considered: either TC affects the membranes of bone cells concerning the transport of calcium[9] or/and the loss of both components through the kidneys. We are well aware of the fact that kidneys are not looked upon as a target organ, which is influenced by TC. But this question is far from being clear. There is no agreement concerning the phosphaturic effect of TC, and even in case of calciuria, the results are perhaps affected by different conditions of the experiment. A decrease of urinary calcium after TC infusion was observed in experimental animals[10], but in normal man TC administration in a single injection enhanced significantly the excretion of calcium[11]. Nothing whatever is known about citric acid excretion.

It was established that factors – such as parathormone and vitamin D – which control the metabolism of calcium,

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Table I

Time	No. of animals	TC	Calcium in serum (mg/100 ml)	Citric acid in serum (mg/100 ml)
Controls (min)	6	Ø	10.28 ± 0.18	5.60 ± 0.17
20	6	+	9.68 ± 0.11[a]	5.46 ± 0.15
40	6	+	8.63 ± 0.22[b]	5.34 ± 0.14[a]
60	12	+	8.39 ± 0.22[b]	4.96 ± 0.20[b]
120	6	+	8.32 ± 0.22[b]	5.10 ± 0.35[a]
180	6	+	9.00 ± 0.30[b]	5.35 ± 0.21[a]

Control animals received 1 ml of acetate buffer pH 3.8. Experimental animals received 8 units according to Hirsch[2]: approximately 0.8 MRC units. [a] Statistical significance on the 5% level. [b] Statistical significance on the 1% level.

Table II

	No. of animals	TC	Calcium in serum (mg/100 ml)	Citric acid in serum (mg/100 ml)	Magnesium in serum (mg/100 ml)
Controls Single dose (min)	4	Ø	9.80 ± 0.12	5.60 ± 0.19	4.20 ± 0.12
60	4	12 Hirsch U 1.2 MRC U	7.85 ± 0.27[a]	4.00 ± 0.13[a]	4.07 ± 0.10
180	4	12 Hirsch U 1.2 MRC U	8.72 ± 0.18[a]	5.30 ± 0.30	4.54 ± 0.22
Repeated dose (min)					
60	5	4 × 3 Hirsch U	6.96 ± 0.22[a]	4.85 ± 0.07[a]	4.40 ± 0.16

Control animals received 3.8 ml of acetate buffer pH 3.8. In case of the repeated dose TC was injected 4 times in the intervals of 30 min and the animals were sacrificed 60 min after the last injection. [a] Indicates the statistical significance on the 1% level.

also intervene actively in the metabolism of citric acid[12,13]. Accumulation of citric acid in kidneys and bones was observed after administration of parathormone or vitamin D. We have therefore examined in our experiments the concentration of citric acid and calcium in the kidneys and bones, but the experimental group showed no difference in comparison with the controls. Only the content of calcium in the kidneys was slightly diminished in the experimental group.

So it appears that after administration of TC the citric acid follows the serum calcium level and the quantity of ionized calcium very likely does not change, but no proof has been obtained that TC interfers with the metabolism of citric acid.

Zusammenfassung. Thyreocalcitonin, das neue Kalzium und Phosphor senkende Hormon, wurde auf seine Wirkung auf den Zitronensäuregehalt von Plasma, Nieren und Knochen untersucht. Dabei wurde eine Senkung der Zitronensäure im Plasma festgestellt, nicht aber eine solche in Knochen und Niere.

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Chromosome Morphology of Sceloporine Lizards (*Sceloporus occidentalis* and *S. graciosus*)

Cytogenetic techniques for the examination of somatic chromosomes of mammalian cells have recently been extended to reptilian studies. Because of these advances, it has been possible to obtain karyotypes of several representative reptiles to date. Some important points about the reptilian chromosomes have been described in these studies such as the evolutionary patterns of the order crocodilia[1] and sex chromosomes within the microchromosome portion of a karyotype[2]. In none of these studies has a nucleolus organizer region been described in a reptilian karyotype. Our recent studies of chromosomes from 2 lizard species indicate the consistent presence of satellited chromosomes within the karyotype. This report describes their appearance and behavior in our tissue cultured cells from *Sceloporus occidentalis* and *S. graciosus* collected from the Laguna mountains in Eastern San Diego County, California.

Myocardial tissue was minced and placed in milk dilution bottles in Eagles media supplemented with 2× extra amino acids and containing 20% fetal calf serum and penicillin and streptomycin. The cultures were incubated at 30 °C, and after several days the tissue pieces attached to the bottle, and cellular outgrowth began. After 2–3 weeks, the cells were removed from the glass surface with trypsin (0.25%) and transferred to new bottles. Thereafter, they are handled in the same manner as mammalian cell cultures with the exception of the incubation temperature. Chromosome preparations are generally made on the third day after passage when the cells are incubated with 0.2 μg/ml colcemid for 6 h and then harvested by trypsinization. The cells are treated with 0.075*M* KCl for 17 min, fixed in 3:1 ethanol-acetic acid, and air-dried on a glass slide. Some cultures have been maintained for over one year to date, and although their mitotic activity is low when compared to mammalian cell cultures, they have remained predominantly diploid.

The karyotypes of both species show 12 macrochromosomes in the several individuals of both sexes which were examined. The karyotypes are shown in Figure 1 and show *S. occidentalis* to have 12 macrochromosomes and 10 microchromosomes; whereas *S. graciosus* has 12 macrochromosomes and 18 microchromosomes. We were unable to see any difference between the 2 sexes in either species, confirming COLE et al.[3] in their report on *S. occidentalis*.

There are 2 principal observations of interest which can be made in the karyotypes of these 2 species. First, it is evident that both have similar macrochromosome morphology. In contrast to the arrangement of LOWE et al.[4], it seems more logical to arrange the largest 6 pairs of chromosomes from both species as macrochromosomal elements. In fact, a review of published karyotypes of other 'Sceloporine' species[2,5–7] show essentially identical macrochromosome morphology for representatives of the genuses Uta, Uma and Sceloporus. However, only LOWE's karyotype of *S. magister* shows satellited chromosomes in the complement. Our preparations from *S. graciosus* have consistently had satellites of the long arm of what we choose to call chromosome pair No. 2. The appearance of these satellites varies from preparation to preparation and spread to spread as is true for satellites in other animal (Figure 1a) karyotypes. Our first preparations from *S. occidentalis* showed no satellites (Figure 1b) but consistently demonstrated a high incidence of long-arm telomere association of pair No. 2 (Figure 2a, b). We believed that this phenomenon suggested the presence of non-visualized satellites or satellites in an altered morphological and functional state. Subsequent preparations from these serial cultures have confirmed this by demonstrating satellites on this chromosome pair (Figure 2c). Satellite presence is classically associated with nucleolar organizing function. The variable appearance probably corresponds to different functional states of the organizer. After seeing our satellited preparations, COHEN[8] reported

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