of this age to ascertain the changes in the nature of the several cellular components that figure importantly in uterine growth[11].

Riassunto. Si dimostra che l'utero di ratta, sottoposta ad ablazione della ovaie 15 mesi e mezzo prima, risponde nettamente a tre iniezioni sottocutanee quotidiane di 17β-estradiolo: si ottennero aumenti di peso del 92, 207 e 231% al di sopra del peso degli uteri di controlli sottoposti ad ovariectomia e trattati con semplice olio di sesamo. Si conclude, percio, che l'utero di ratta esposta a deficienza ormonica ovarica di lunga durata è notevolmente sensibile a trattamento con estrogeni.

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Evidence for a Hypocalcemic Factor in the Hypothalamus

The hypocalcemic effect of the pituitary gland extracts have been shown by several workers[1-5]. So far, there is no convincing evidence that the hypothalamus is concerned in the production of hypocalcemia. The present study suggests the existence of a hypocalcemic factor in the hypothalamus.

Material and methods. 72 albino rats of Hacettepe strain, weighing 150 to 180 g and 470 guinea-pigs were used in this study. All rats were placed on a special low calcium diet for 3 days before the experiment. Fragments of hypothalamic tissue, containing mainly pituitary stalk and median eminence (SME), and cerebral cortex in an equivalant amount to SME were removed from 470 guinea-pigs immediately after decapitation, frozen on dry ice and were kept for a maximum of 24 h. The 2 pools, SME and cerebrum, were thawed and each one homogenized in 25 ml chilled 0.9% saline solution. The crude homogenates were then subjected to centrifugation at 11.000 *g* for 5 min at 4 °C and the supernatants were separated. The procedures were repeated once more with 15 ml of normal saline and total extracts were kept at −20 °C. The extracts were used within 10 days.

Hypophysectomy was performed according to the method of FALCONI et al.[6]. The experiment was started the day after hypophysectomy. Rats were anesthetized with i.p. pentobarbital, 30 mg/kg of body weight prior to the experiments, after which tracheostomy was performed. A fine polyethylene catheter was inserted into the right jugular vein for extraction of blood. Brain or SME extracts or saline was injected through the left carotid artery. 1 mg of heparin was administered i.v. to each animal to prevent clotting of the blood. 1 ml of blood was drawn at O 'base line' after 20 and 30 min and in 1 experiment at 60 min for calcium determination. Each time fluid loss was replaced by infusing normal saline. Plasma calcium was determined by the method of REHELL[7]. All samples were run in duplicate.

The following 4 different experiments were carried out in this study: Experiment I 27 rats were used in this experiment. First 1 ml of blood was obtained from each intact rat and then the animals received a single 0.5 ml SME extract/100 g body wt. by carotid artery over 2 min. 15 control rats received only normal saline 0.5 ml/100 g body wt. by carotid artery. 1 ml of blood was drawn at 0, 20 and 30 min for Ca determination.

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Plasma Ca levels before and after injection of SME extract or physiological saline

Experiment	No. of rats	Infused solutions	Plasma Ca levels mg/100 ml (mean ± SE)			
			Preinjection (min)		Postinjection (min)	
			0	20	30	60
I	27	Hypothalamic (SME) extract	10.48±0.081	8.37±0.182 P<0.001[a]	8.35±0.178 P<0.001[a]	—
	15	Physiologic saline	10.39±0.100	10.10±0.090 P>0.05	10.19±0.094 P>0.05	—
II	10	Cerebral Cortex	10.94±0.168	—	10.92±0.162 P>0.05	10.97±0.189 P>0.05
III	10	Hypothalamic (SME) extract	10.78±0.288	9.56±0.302 P<0.001[a]	9.19±0.320 P<0.001[a]	—
IV	5	Heated (SME) extact	10.80±0.423	10.88±0.312 P>0.05	10.82±0.353 P>0.05	—
	5	Digested (SME) extract	11.00±0.221	10.60±0.299 P>0.05	10.62±0.312 P>0.05	—

a — $P < 0.001$ as compared with saline infused control.

Experiment II. 10 rats were used. After 1 ml of blood extraction, the equivalent amount of cerebral cortex extract to SME extract was infused into the rats by carotid artery, as in experiment I, over 2 min. 1 ml of blood was drawn at 0, 30 and 60 min for Ca determination.

Experiment III. 10 rats were used. Stalk Median Eminence extract, 0.5 ml/100 g body wt. was infused to hypophysectomized rats. The same procedures were used as in experiment I. 1 ml of blood was drawn at 0, 20 and 30 min for Ca determination.

Experiment IV. A part of SME extract was heated for 5 min in boiling water, another part was subjected to tryptic digestion according to the method of LASCOWSKI[8]. The amount of the SME extract and the same procedures were used as in experiment I. 1 ml of blood was drawn at 0, 20 and 30 min for Ca determination

The difference between the means were tested by Student's t test[9].

Results. Hypothalamic extract produced a striking fall in plasma calcium of intact and hypophysectomized rats (experiments I and III). The fall in mean plasma calcium levels, as compared with saline infused control, are statistically significant, $P < 0.001$ (Table). There was no significant fall in plasma calcium levels in experiment II, $P > 0.05$ (Table). Boiling or tryptic digestion abolished the hypocalcemic effect of the hypothalamic extract $P > 0.05$ (Table).

Discussion. We have demonstrated in a previous study[4] that the pituitary extract obtained from guinea-pigs immediately after decapitation shows a plasma calcium lowering effect, whereas pituitary extract from guinea-pigs, obtained 24 h after the pituitary stalk section, has no such activity[10]. On the other hand, extracts of the SME prepared from the guinea-pigs 24 h after pituitary stalk section lowers plasma calcium in rats[10]. The present study also shows that the SME extract obtained from guinea-pigs immediately after decapitation produces a hypocalcemic effect in both intact and hypophysectomized rats. Namely the hypocalcemic affect of the SME extract is evident either in the presence or in the absence of the pituitary gland. The above findings lead us to believe that the pituitary hypocalecemic factor is probably produced in the hypothalamus and then moved into the pituitary gland to be stored there. When the pituitary stalk is sectioned the hypophyseal depot is exhausted. No hypocalcemic activity could be demonstrated in the brain tissue extract of guinea-pigs which were injected into the bioassay rats. The phosphorus content of the hypothalamus was not related to its hypocalcemic effect since an equivalent amount of phosphorus to SME extract phosphorus produced no significant fall in blood calcium level when injected to rats[10].

Loss of activity by boiling or tryptic digestion may suggest that the hypothalamic factor is a protein or a polypeptide.

Zusammenfassung. Es konnte gezeigt werden, dass ein Hypothalamus-Extrakt von Meerschweinchen an hypophysektomierten Raten eine deutliche Senkung des Plasmakalziums bewirkte.

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Biological Activity of a Synthetic Decapeptide Corresponding to the Proposed Growth Hormone-Releasing Hormone

The isolation in our laboratory of a proposed growth hormone-releasing hormone (GH-RH) from porcine hypothalami[1] was followed recently by determination of the structure of this decapeptide[2] and its synthesis[3]. This paper summarizes the results of biological tests carried out on the synthetic decapeptide.

Materials and methods. Synthetic Val-His-Leu-Ala-Glu-Glu-Lys-Glu-Ala[1] and Val-His-Leu-Ser-Ala-Glu-Glu-Lys-Gln-Ala (II) were prepared by VEBER et al.[3]. The synthetic decapeptide (I) was indistinguishable from natural GH-RH[1-3]. The GH-RH activity in vitro was assayed by the methods of SCHALLY et al.[4] and/or of DICKERMAN et al.[5]. The GH released was measured by the 'tibia test' of GREENSPAN[6]. The GH was also measured by in vivo formation of sulfation factor (stimulation of ^{35}S incorporation into costal cartilage of hypophysectomized rats)[7] as well as by radioimmunoassay (RIA) for rat GH[8], using NIAMD-RAT-GH RIA kit.

Results. When synthetic GH-RH was added to the incubation medium in vitro in picogram (pg) doses, the release of GH was stimulated when measured by the tibia test (Table I) or by formation of sulfation factor activity (Table II). Similar results were observed in at least 10 other experiments. The magnitude of stimulation of GH release determined by these 2 methods, using the same samples was not identical. No explanation for this discrepancy is available at present. The GLN-9-GH-RH (II), which was found as a fraction of the isolated material[2,3], also had some GH-RH activity in vitro. The results in Table I indicate that the dose response regression lines for natural and synthetic GH-RH preparations were parallel and the potency of the synthetic GH-RH was

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