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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Table I. The effect of natural and synthetic GH-RH on the release of GH from rat pituitaries in vitro

Group	pg GH-RH/ pituitary	Bioassay for GH by the 'tibia test'		RIA for rat GH μg GH[d] Total
		Average tibial witdth μm (mean ± SE)[a]	GH released μg[b, c] Total	
Natural GH-RH	50	144.4±5.3	64.1 (37.3– 110.1)	25
	250	170.6±5.6	320.3 (186.4– 550.2)	77
	100	146.5±5.5	89.9 (50.7– 142.2)	22
Synthetic GH-RH	500	179.0±0.8	424.4 (253.3– 710.8)	153
	2500	202.0±3.1	1273.2 (760.1–2132.6)	18

[a] Hypophysectomized assay control = 129.9±3.3, 20 μg GH = 157.4±4.0; 100 μg GH = 188.4±5.4; 300 μg GH = 213.1±4.4. [b] Mean and 95% confidence limits. [c] Expressed as μg equivalents of NIH-GH-S8. Relative potency synthetic versus natural GH-RH = 0.66 (0.39–1.11). Index of precision of the assay $\lambda = 0.24$. [d] Expressed as NIAMD-RAT-GH-RP-1.

Table II. In vivo ^{35}S incorporation into costal cartilage of hypophysectomized rats after administration of incubation medium from pituitaries stimulated with natural and synthetic GH–RH

Group Treatment	Dose of GH–RH in vitro (ng/ml)	^{35}S incorporation cartilage ± S.E. (dpm/mg)	GH[a] (μg)	P
1 Control medium	—	855±117 (6)[b]	12	—
2 Natural GH–RH	1	1456± 84 (10)	36	2 vs 1 0.001
3 Natural GH–RH	5	1533± 65 (7)	37	3 vs 1 0.001
4 Synthetic GH–RH (I)	2	1401± 98 (9)	32	4 vs 1 0.005
5 Synthetic GH–RH (I)	10	1538± 73 (7)	37	5 vs 1 0.001
6 Growth Hormone std[a] (20 μg)	—	1045± 98 (8)	—	6 vs 1 NS
7 Growth Hormone std[a] (180 μg)	—	2761±183 (10)	—	7 vs 1 0.001
8 Hypophysectomized Control	—	400± 18 (10)	—	8 vs 1 0.001

[a] As NIH–GH–S–8. [b] Number of cartilages used.

approximately one half of that of the postulated natural GH–RH. In contrast, when the incubation medium was assayed by the RIA for rat GH, no consistent effects on GH release could be detected (Table I).

Discussion. The GH–RH isolated from porcine hypothalami was previously shown to deplete the pituitary content of GH in rats[1], to stimulate GH-release and synthesis in vitro[1,9] and to accelerate the extrusion of GH-containing granules from somatotrophs of the rat pituitary[10]. Recently, we reported that the natural preparation of GH–RH increased plasma GH-like activity in rats[11]. This paper demonstrates that a synthetic decapeptide corresponding in structure to natural GH–RH stimulated the release of GH in vitro as measured by the tibia test[6] or by sulfation factor assay. DAUGHADAY et al.[12] reported that sulfation factor is not the growth hormone itself, but a substance in rat serum, which is generated by GH. The activity of synthetic GH-RH could not be demonstrated in vitro by the radioimmunoassay for rat GH. Although depletion of pituitary GH content was observed in vivo in rats after administration of the synthetic decapeptide, no rise in plasma RIA-GH was seen in rats, sheep, pigs and monkeys[12,13]. The reasons for these discrepancies are not yet apparent since crude hypothalamic extracts or partially purified preparations recently were reported to stimulate the release of RIA-GH in vivo and in vitro in the rat[13–16]. It is possible that in the absence of a cofactor which may be present in crude hypothalamic preparations, pure natural GH-RH or the synthetic decapeptide with a corresponding structure cannot affect the release of radioimmunoassayable GH. In view of the aforementioned findings which remain unexplained, it would appear prudent to hold in abeyance any definitive conclusion as to whether the polypeptide in question[1–3] is the true growth hormone-releasing hormone, responsible for regulation of GH from the pituitary gland under physiological conditions. Further studies are needed to shed more light on this difficult problem[17].

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Résumé. Le décapeptide synthétique Val-His-Leu-Ser-Ala-Glu-Glu-Lys-Glu-Ala, dont la structure correspond à celle proposée pour la hormone hypothalamique de libération de l'hormone de croissance hypophysaire (GH-RH) a été étudié in vitro pour contrôler l'activité de la GH-RH. Le décapeptide synthétique a stimulé la sécretion de l'hormone GH comme on a pu le constater par des dosages biologiques. L'utilisation du système radioimmunologique pour le dosage de la GH n'a pas fait apparaître cette augmentation de décharge de GH dans l'hypophyse, en présence de la GH-RH d'origine naturelle ou du décapeptide synthétique.

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Effect of Pregnancy and Feeding Pattern on Tryptophan Pyrrolase in the Rat

The activity of liver tryptophan pyrrolase in the pregnant rat has been reported to be increased on the 13th day of pregnancy[1] and to be unchanged from normal levels on the 19th day[2]. The ability of glucocorticoids to raise the level of liver tryptophan pyrrolase is well-established[3,4] and the increase in this enzyme in certain conditions of of stress[5,6] and following oestrogen administration[7] has been attributed to adrenal cortical activity. In the pregnant rat there is no evidence of an increase in plasma oestrogen until the day preceding partuition[8], the level of circulating corticosterone is depressed from the 8th day of pregnancy onwards[9] and the ability of the liver to inactivate corticosterone is increased from the 12th day[10]. The present work was therefore undertaken to study more fully the activity of tryptophan pyrrolase in the rat during pregnancy and to investigate the response of the enzyme to different feeding patterns in the pregnant rat.

Methods. Virgin Wistar rats of fasting body weight 180–200 g were mated and along with non-pregnant rats of similar weight allowed free access to commercial rat cake (North Eastern Agricultural Co-operative, Ltd., Aberdeen) or fed daily at 15.00 h 14 g of this rat cake, powdered and made into a paste with water. Rats were killed at 09.00 h unless stated otherwise at various stages of pregnancy, day 1 being the day following the observation of spermatozoa in the vagina. Non-pregnant rats were killed after either 13 or 20 days on the two regimes. Tryptophan pyrrolase activity was assayed essentially as described by Knox, Piras and Tokuyama[11]. Statistical evaluation of results was carried out using Student's *t*-test.

Results and discussion. 13- and 20-day pregnant rats fed freely had significantly greater hepatic tryptophan pyrrolase activity than non-pregnant rats, the increase on the 20th day being significantly greater than that on the 13th day (Table). Pregnant rats voluntarily increase their food intake by up to 30%[12] and the level of tryprophan pyrrolase in normal rats varies with the pattern of feeding and amount of food eaten[13]. Thus the increase observed in tryptophan pyrrolase may be due to variations in dietary habits of the pregnant animals. For this reason the enzyme level in pregnant and control rats was compared when both were fed the same amount of diet as a single meal daily at 15.00 h. On the 8th, 10th and 11th days of pregnancy the level of tryptophan pyrrolase was not significantly different from that in the non-pregnant animals. Except on the 14th day, from the 12th day of pregnancy tryptophan pyrrolase was higher than in the non-pregnant rat with levels between the 16th and 20th days being higher than those on the 15th day or earlier (Figure 1). The fact that normal levels of tryptophan pyrrolase have been previously reported in 19-day pregnant rats[2] may be due to the fact that in these earlier studies the method of enzyme assay used may not have ensured complete activation of all enzyme molecules present. The lower activity of tryptophan pyrrolase on the 14th day of pregnancy may be related to the fact that induction of this enzyme does not occur when liver DNA is being synthesised[14]. Campbell and Kosterlitz[15] found increases in DNA content and a tendency for increased incorporation of $^{32}PO_4$ into DNA in livers of 14-day pregnant rats. Restriction of food intake did not significantly affect the absolute level of enzyme activity on the 13th and 20th days of pregnancy

Tryptophan pyrrolase activity in livers of non-pregnant and 13- and 20-day pregnant rats

Treatment	μmoles kynurenine (g liver/h)	*P* I	*P* II
non-pregnant (9)	3.34±0.38	—	—
13-day pregnant (6)	5.96±0.84	<0.01	—
20-day pregnant (3)	11.17±1.00	<0.001	<0.025

Results are expressed as the mean ± S.E. Numbers in parentheses represent the number of animals in each group. I. Statistical significance of difference between pregnant and non-pregnant groups. II. Statistical difference between the two pregnant groups.

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