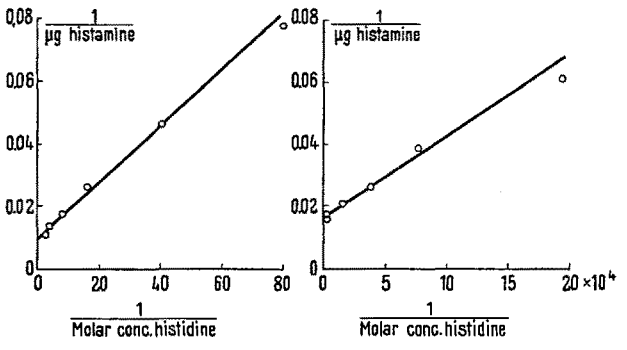
mals[8]. Such an enzyme seems to occur in the mast cells of the rat and mouse[9,10]. From the investigations of KAHLSON et al.[11], it has appeared that some growing tissues have a high histidine decarboxylase activity. This enzyme has been studied and been found to be of another kind than that found in the rabbit kidney. It has thus been found to have a K_m-value of about 2×10^{-5} (Fig.), which is in the same order of magnitude as the K_m of histidine decarboxylase in mast cells[10]. In contrast to the kidney enzyme, it is not strongly inhibited by the DOPA decarboxylase inhibitors α-methyl DOPA or caffeic acid.

The difference between the K_m-values obtained might be explained by the presence of an endogenous, non-dialysable inhibitor in the kidney extract causing an apparently greater K_m. This possibility seems, however, to be excluded, as the decarboxylation in rat liver extracts was not inhibited on addition of large amounts of the rabbit kidney extract.

In conclusion it may be said that mammalian tissues contain at least two different histidine decarboxylating enzymes: one probably being identical with DOPA decarboxylase and supposedly of no importance for the physiological formation of histamine. The other (or others) is located in mast cells and (or) in growing tissues. As the enzymes differ in several respects, due consideration must be taken of this fact when studying the properties of histidine decarboxylating enzyme systemes.

Zusammenfassung. Säugetiergewebe enthalten mindestens zwei verschiedene, Histidin-decarboxylierende Fermente. Das eine, wahrscheinlich mit Dopadecarboxylase identisch, scheint für die Histaminbildung unter physiologischen Bedingungen ohne Bedeutung zu sein. Das andere ist in Mastzellen und wachsenden Geweben lokalisiert.



Effect of substrate concentration on the rate of histamine formation in rabbit kidney cortex extract (to the left) and on the foetal rat liver extract (to the right). The reciprocal of amine formed per g tissue in 3 h is plotted against the reciprocal of histidine concentration.

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Effect of a Fraction of Bovine Hypothalamic Extract on the Release of TSH by Rat Adenohypophyses *in vitro*

It was found in previous experiments[1] that the non-protein fraction of rabbit and bovine hypothalamic extracts activates adenohypophysial acid phosphatases *in vitro*. A relationship was also found between the activity of adenohypophysial acid phosphatases and TSH secretion *in vivo*[2]. The hypothesis that the acid phosphatase-activating factor is the TSH-releasing factor of the hypothalamus was previously tested in experiments *in vivo*, and it was shown[3] that the fraction activates the secretion of TSH from rat adenohypophysial autografts in the anterior chamber of the eye of hypophysectomized rats.

The active non-protein fraction of bovine hypothalamic extract (for preparation[4], lot No. HH_9) was further purified by high-voltage electrophoresis[5]. The zone containing the factor activating adenohypophysial acid phosphatases *in vitro* was eluted by 0.09% NaCl and the subfraction was labelled HH_{9+1}. 1 cm³ of the eluate of this active zone corresponded to 19 mg of the original non-protein fraction HH_9. The effect of the electrophoretically homogenous subfraction HH_{9+1} on TSH release by rat adenohypophyses was tested *in vitro*.

Female albino rats (descendants of the Wistar strain) acclimatized at 23 ± 1°C, fed standard Larsen diet and water *ad libitum*, were killed by decapitation and their adenohypophyses were removed. In each of three successive experiments, 10–13 pooled adenohypophyses were incubated for 1 h at 37 ± 0.1°C in two different media: A) 10 cm³ Krebs-Ringer-phosphate + 3 cm³ 0.09% NaCl; B) 10 cm³ Krebs-Ringer-phosphate + 3 cm³ subfraction HH_{9+1}. Both media contained 300 mg glucose/100 cm³. In both groups and all experiments 6.6 mg adenohypophysial tissue was incubated in 1 cm³ medium. After incubation the media were separated and frozen.

The amount of TSH in the media was estimated by the radio-iodine uptake method, described elsewhere[6]. Female albino Wistar rats weighing about 100 g were hypophysectomized by the parapharyngeal route under ether anaesthesia. Three days after hypophysectomy, administration of the test material was started; once daily every rat received one subcutaneous injection of 0.5 cm³. Four groups of rats were used:
(1) 16 hypophysectomized rats without any injection served as controls; (2) 14 hypophysectomized rats received four doses of 0.5 cm³ of the mixture of 10 cm³

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Group	1	2	3	4
Administered material	0	medium with fraction HH_{9+1}	medium after incubation of hypophyses without HH_{9+1}	medium after incubation of hypophyses with HH_{9+1}
Number of tests	16	14	14	15
Concentration of fraction $HH_9{}^+{}_1$ in the medium	—	1 cm³/3.3 cm³	0	1 cm³/3.3 cm³
Total dose of tested material in cm³	0	2	2	2
Mean weight of adenohypophyses incubated in 1 cm³ medium in mg	—	—	6.6	6.6
% of the dose of radio-iodine found in the thyroid gland. Means $\pm \sigma_m$	0.9 ± 0.10	1.3 ± 0.18	2.2 ± 0.33	5.1 ± 0.87 [a]

[a] Comparison of groups 3 and 4 by Fisher's t-test: $p < 0.01$

Krebs-Ringer-phosphate and 3 cm³ fraction HH_{9+1} (second control group); (3) 14 hypophysectomized rats received four doses of 0.5 cm³ of medium A (after incubation of the hypophyses without fraction HH_{9+1}); (4) 15 hypophysectomized rats received four doses of 0.5 cm³ of medium B (after incubation of the hypophyses with fraction HH_{9+1}).

1 h after the last injection 4 μC of carrier-free $Na^{131}I$ was administered to each rat subcutaneously. 24 h after the administration of radio-iodine, the rats were killed by ether anaesthesia, the thyroid glands were removed and their radioactivity was measured by Geiger-Müller counter. The activity of each thyroid was expressed as a percentage of the radio-iodine dose administered, a model of the rat thyroid containing 4 μC $Na^{131}I$ being used as the indicator of 100% accumulation.

The results are shown in the Table. The radio-iodine uptake in rats of group 2 was not statistically different from group 1, indicating that no TSH was present in fraction HH_{9+1}. After the administration of medium A (after incubation of the adenohypophyses without fraction HH_{9+1}), the radio-iodine uptake doubled, indicating some release of TSH into the medium. After the administration of medium B, a substantial elevation in thyroid radio-iodine uptake was registered, idincating an at least two-fold increase in TSH release into the medium in the presence of fraction HH_{9+1}. It appears, therefore, that fraction HH_{9+1} stimulated the release of TSH from rat adenohypophysial tissue *in vitro*. This is in agreement with our previous results on the effect of the original non-protein fraction HH_9 on TSH release from adenohypophysial autografts *in vivo*[3]. Further work is needed to prove the specificity of this reaction and to test whether only release or also the production of TSH is influenced.

Zusammenfassung. Die elektrophoretisch isolierte Fraktion des hypothalamischen Extraktes erhöht *in vitro* die Sekretion des thyreotropen Hormons aus der Adenohypophyse der Ratte in der Krebs-Phosphat-Ringerlösung mit 300 mg% Glukose. Die Fraktion selbst besitzt keine TSH-Wirkung.

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Influence of the Structure of the *N*-terminal Extremity of α-MSH on the Melanophore Stimulating Activity of this Hormone

We have formerly reported[1,2] the total synthesis of α-MSH[3,4]. When compared with natural hog α-MSH[5], our synthetic material has shown the same melanophore stimulating activity by both the *in vivo*[6] and the *in vitro*[7] assays[8,9], the same kinetics of degradation by chymotrypsin and by trypsin, and the same chromatographic and electrophoretic behaviour under a variety of conditions, thus definitely proving its complete identity with the natural product. We have also found that our synthetic product loses its biological activity under the action of hydrogen peroxyde and regains it almost completely by subsidiary treatment with cysteine or thioglycollic acid[10].

This successful synthesis of α-MSH opened the way for obtaining synthetic analogues of this hormone. By following a scheme of synthesis similar to that one we used for α-MSH, we have prepared the three structural analogues (A, B, and C) mentioned in the Table, in order to investigate the influence of the structure of the *N*-terminal extremity on the biological properties[11].

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