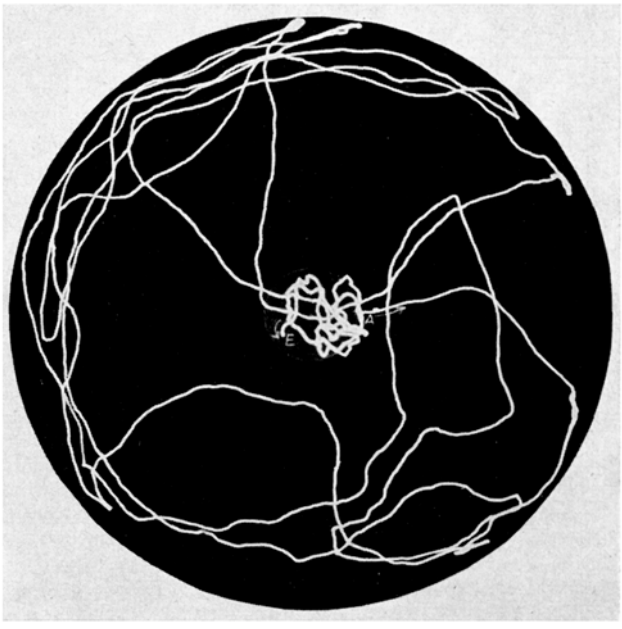

Fig. 3. Bastard *Tilapia mossambica* ♂ × *Tilapia tholloni* ♀. 300 sec. Alter 21 Tage. Sonst wie bei Fig. 1.

Die vorgelegten Beispiele sind typisch. Es wurden insgesamt 54 Einzelversuche mit 11 *mossambica* angestellt, 79 Versuche mit 12 *tholloni* und 121 mit 20 Bastarden. In keinem Falle äusserten die jungen *tholloni* Kontaktverhalten; *bei jedem der Versuchstiere der beiden anderen Gruppen konnte es mindestens während einer Phase von etwa 10 Tagen erzielt werden.* Übrigens waren die Bastarde Halbgeschwister der *tholloni*-Kontrollen und stammten aus dem gleichen Gelege. Ein Teil der als Kontrollen verwendeten *mossambica* hatte den gleichen Vater wie die Bastarde.

Summary. The young of *Tilapia mossambica* (mouthbrooder) show a characteristic 'contact-behaviour', which is related to the behaviour of the mother fish, who takes them back into the mouth cavity in cases of disturbance. This contacting is lacking in the young of *Tilapia tholloni* (substrate spawner). Contacting, as studied in our experiments with models, is genetically determined and is dominant over the *tholloni* pattern in the cross *T. mossambica* ♂ × *T. tholloni* ♀.

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[4] Mit Unterstützung durch die Deutsche Forschungsgemeinschaft.

On the Presence of Different Histidine Decarboxylating Enzymes in Mammalian Tissues

In a previous paper, the histidine decarboxylase found in rabbit kidney cortex has been reported to be identical with L-3,4-dihydroxyphenylalanine (DOPA) decarboxylase[1]. At about the same time, Udenfriend, Lovenberg, and Weissbach[2] reached an analogous result working with guinea-pig kidney extracts. Some additional results concerning this problem will be given here.

The enzyme preparations used were obtained from rabbit kidney cortex or foetal rat liver. The tissues were extracted according to Rosengren[1]. The enzymic material was precipitated with ammonium sulphate and dialysed against distilled water at 4°C over-night. The extracts were then incubated in an atmosphere of nitrogen at 37°C and pH 7.8. The histidine decarboxylase activity was estimated by measuring the amount of histamine formed in 3 h. The extraction and purification procedures of histamine were principally the same as those described for catecholamines[3]. The final estimation of histamine was carried out fluorimetrically according to Shore, Burkhalter, and Cohn[4]. A detailed account of the method used will be given elsewhere.

In some experiments, the effect of various substrate concentrations on the rate of histidine decarboxylation in rabbit kidney cortex extracts was studied. The results obtained are given in the Figure. As will be seen from this plot, K_m is very great—about 10^{-1}. This finding raised the interesting question if this enzyme is really responsible for the formation of histamine from histidine in the body under physiological conditions. The reaction appears, indeed, to be effected by DOPA decarboxylase, which is known to be present in large amounts in the rabbit kidney. This opinion is supported by the following observations: 1. The formation of histamine is inhibited by DOPA and by DOPA decarboxylase inhibitors, e.g. caffeic acid, catechol, α-methyl DOPA[5] as observed by us as well as by others[2,6,7]. 2. The decarboxylation of DOPA in kidney extracts is inhibited by addition of large amounts of L-histidine. In a typical experiment 25 μg dopamine was formed in an incubation mixture containing 0.1 ml purified rabbit kidney cortex extract and 200 μg DOPA after 5 min. If 50 mg L-histidine was added together with 200 μg DOPA and incubated under identical conditions, only 14 μg dopamine was formed in the same time. The degree of inhibition is of the order of magnitude that could be expected if the two activities are achieved by one enzyme. 3. The two activities could not be separated during fractionation by means of precipitation with ammonium sulphate or chromatography on a DEAE cellulose column.

The question now arose whether there was another mammalian enzyme that could decarboxylate histidine, as this reaction has been shown to occur after injection of very small amounts of histidine into experimental ani-

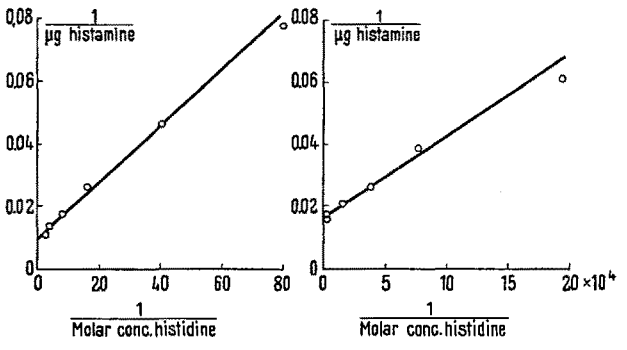
[1] E. Rosengren, Acta physiol. scand. *49*, 364 (1960).
[2] S. Udenfriend, W. M. Lovenberg, and H. Weissbach, Fed. Proc. *19*, 7 (1960).
[3] Å. Bertler, A. Carlsson, and E. Rosengren, Acta physiol. scand. *44*, 273 (1958).
[4] P. A. Shore, A. Burkhalter, and V. H. Cohn, J. Pharmacol. exp. Therap. *127*, 182 (1959).
[5] It is not possible at present to decide if the inhibition of α-methyl DOPA is due to an interference between this substance and histidine at the active center of the enzyme or lack of pyridoxal-5-phosphate. It was found that, under the conditions prevailing, α-methyl DOPA readily reacts with pyridoxal-5-phosphate as determined spectrophotometrically.
[6] E. Werle, Biochem. Z. *311*, 270 (1942).
[7] D. Mackay and D. M. Shepherd, Brit. J. Pharmacol. *15*, 552 (1960).

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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Department of Pharmacology, University of Lund (Sweden), January 20, 1961.

[8] S.-E. LINDELL and R. W. SCHAYER, Brit. J. Pharmacol. *13*, 89 (1958).
[9] R. W. SCHAYER, Amer. J. Physiol. *186*, 199 (1956).
[10] P. HAGEN, N. WEINER, S. ONO, and FU-LI LEE, J. Pharmacol. exp. Therap. *130*, 9 (1960).
[11] G. KAHLSON, E. ROSENGREN, H. WESTLING, and T. WHITE, J. Physiol. *144*, 337 (1958).
[12] This work has been supported by grants from the Swedish Medical Research Council.

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

[1] V. SCHREIBER, Acta univ. carol. med. (Prague), in press.
[2] V. SCHREIBER and V. KMENTOVÁ, Endokrinologie (Leipzig) *38*, 69 (1959).
[3] V. SCHREIBER, M. RYBÁK, and V. KMENTOVÁ, Exper. *16*, 466 (1960).
[4] V. SCHREIBER, M. RYBÁK, and V. KMENTOVÁ, Physiol. Bohemoslov. *9*, 303 (1960).
[5] V. SCHREIBER, J. KOČÍ, A. ECKERTOVÁ, and V. KMENTOVÁ, Physiol. Bohemoslov., in press.
[6] A. ECKERTOVÁ and Z. FRANC, Čsl. fysiologie (Prague) *10*, 48 (1961).