various locations along the major curvature (to establish their regional distribution); or in 20 μ horizontal serial sections (to establish their topographical distribution).

The regional distribution of gastric vitamin B_{12}-binding proteins was found to follow the distribution of the enterochromaffin-like cells in mouse, rat and hamster very closely (Figure 1 and Ref. [10]). In these species, enterochromaffin cells are almost exclusively found in the pyloric gland area, whereas the enterochromaffin-like cells occur in the oxyntic gland area.

Also in the guinea-pig, the distribution of the vitamin B_{12}-binding proteins was well correlated with that of the enterochromaffin-like cells, which occurred in the pyloric gland area and in the adjacent portion of the oxyntic gland area (Figure 1, see also Ref. [17]). There was no correlation between vitamin B_{12}-binders and chief cells.

The rabbit was exceptional in that the distribution of the vitamin B_{12}-binging proteins closely agreed with a rich population of markedly reserpine-resistant 5-HT-containing enterochromaffin cells (referred to as enterochromaffin cells, Type II[17]) in the oxyntic gland area (Figure 1), which is poor in enterochromaffin-like cells[17]. It could be shown that in this region the vitamin B_{12}-binging proteins were restricted to the basal layer of the mucosa, a topographical distribution which corresponds closely to that of the enterochromaffin cells (Figure 2).

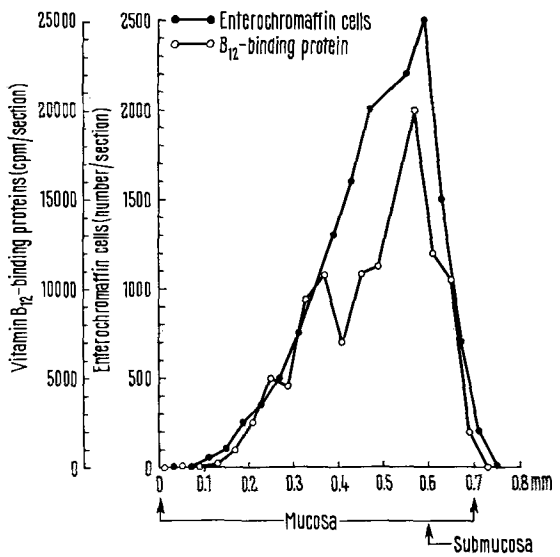

Fig. 2. Topographical distribution of B_{12}-binders and enterochromaffin cells in the oxyntic gland area of the rabbit. Serial sectioning. Each cryostat section (— 36 °C) 20 μ thick, 4 × 4 mm. The content of vitamin B_{12}-binding protein in single sections was determined as described elsewhere[10]. Each value is the mean of 4 experiments.

A high vitamin B_{12}-binding capacity is a basic requirement for IF activity[24,25]. It should be noted, however, that vitamin B_{12}-binders other than IF have been recognized in both gastric juice and gastric mucosa[24-29]. The vitamin B_{12}-binding capacity is thus not by itself a measure of IF content; the estimation of IF levels must be based on the capacity of gastric vitamin B_{12}-binders to facilitate the intestinal absorption of cyanocobalamin. This will be the subject of a separate study.

It is evident that gastric vitamin B_{12}-binding proteins are not regularly associated with one particular region of the stomach and that no previously proposed cell type can be the single cellular storage site for such proteins. It is suggested that the enterochromaffin-like cells in the mouse, rat, hamster and guinea-pig, and the enterochromaffin cells in the rabbit contain gastric vitamin B_{12}-binding proteins. This may indicate some functional similarity between these cells inspite of species-dependent differences as regards their amine storage mechanisms[30].

Zusammenfassung. Es wird angenommen, dass in Maus, Ratte, Hamster und Meerschweinchen Vitamin B_{12}-bindende Proteine sich mit enterochromaffinähnlichen (argyrophilen, nichtargentaffinen) Zellen und im Kaninchen mit einer bestimmten Art enterochromaffiner (argentaffiner) Zellen verbinden.

R. Håkanson, G. Liedberg and K. Lindstrand

Departments of Pharmacology, Histology, Surgery and Hematology, University of Lund, Lund (Sweden), 5 November 1969.

[24] R. Gräsbeck, Acta med. scand. Suppl. *315* (1956).
[25] G. B. J. Glass, Physiol. Rev. *43*, 529 (1963).
[26] L. Ellenbogen and D. R. Highley, J. biol. Chem. *242*, 1004 (1967).
[27] L. Ellenbogen and D. R. Highley, J. biol. Chem. *242*, 1010 (1967).
[28] R. Gräsbeck, K. Simons and J. Sinkkonen, Annls. Med. exp. Biol. Fenn. *40*, Suppl. 6 (1962).
[29] H. Flodh, B. Bergrahm and B. Odén, Life Sci. *7*, 155 (1968).
[30] Supported by grants from the Swedish Medical Research Council (No. B70-14X-1007-05B and No. B70-19X-766-05B) and Albert Påhlsson's Foundation.

5-Hydroxytryptophan-Decarboxylase Activity in the Decapod Crustacean *Upogebia littoralis*

Serotonin is widely distributed in the animal kingdom. All major groups of animals have been found to contain this amine[1].

The occurrence and the physiological role of serotonin in crustacea is very little known, although the plexuses of nerve fibres and the pericardial organs of certain decapods crustaceans have been known to contain appreciable amounts of serotonin[1,2]. More recently, Elofsson et al.[3] have found one pair of large yellow fluorescing cells, which indicate the presence of serotonin in the 'brain' of the crayfish *Astacus astacus*, while later, the same authors[4] could not detect a yellow fluorescence in the nerve fibres of the hind gut.

For a better understanding of serotonin role in crustacea, more information must be obtained about its distribution in various tissues of different species. In the present study, we have undertaken the further investigation of this problem by measuring the activity of serotonin-producing enzyme in various tissues of the abovementioned decapod crustacean.

Materials and methods. The animals (*Upogebia littoralis*) were killed by deep freezing and the organs to be studied were quickly removed. After homogenization in a poter (Elvehjem homogenizer with equal volume of phosphate buffer 0.066 *M*, pH 7.2 and 0.1 *M* cysteine) the homogenate was centrifuged at 13,500 *g* for 30 min. The supernatant was used as enzyme preparation. The reaction mixture consisted of 0.5 ml homogenate, 0.04 μC DL[^{14}C]-5-HTP (s.a. 2 mC/m*M*), 90 nMol non-isotopic DL 5-HTP, in a volume of 0.3 ml, 100 μg pyridoxal phosphate, 10^{-3} *M* isoniazid brought to a final volume of 2.5 ml with 0.066 *M* phosphate buffer pH 7.2. This mixture was incubated for 30 min at 37 °C[5]. After this, the mixture was removed from the water-bath and proteins were precipitated by addition of 5 vol. of cold ethanol. The precipitate was filtered off and the filtrate evaporated in a Rotavapor. The residue was dispersed in water to a volume of 7 ml and mixed with 35 ml of 2:1 v/v chloroform-methanol, to remove the lipids.

For the determination of 5-HTP decarboxylase, a radiochemical test, similar to that described by us previously[6], was used. The determination is based on the conversion of [^{14}C]-5-HTP to [^{14}C]-serotonin, separation of radioactive metabolites by paper chromatography and measurement of the radioactivity of the metabolites directly on the paper by liquid scintillation counting. Proteins were determined by the method of LOWRY et al.[7].

Enzyme activity is expressed as units of enzyme per mg of protein from the obtained percentage of the transformation of [^{14}C]-5-HTP to [^{14}C]-serotonin according to the following equation:

$$\text{Specific activity} = \frac{T}{100\,tm}\left[\frac{b}{s.a.} + \frac{MV}{1000}\right]$$

where, *T*, % transformation of [^{14}C]-5-HTP to [^{14}C]-serotonin; *t*, incubation time; *m*, mg of protein; *b*, μC of [^{14}C]-5-HTP added; *s.a.*, specific activity of [^{14}C]-5-HTP; *M*, molarity of non-isotopic 5-HTP solution; *V*, volume of total added substrate.

Results and discussion. The activity of 5-HTP decarboxylase in the various organs of the decapod crustacean *Upogebia littoralis* is shown in the Table.

The activity of 5-HTP decarboxylase in some organs of *Upogebia littoralis*

Organs	% transformation of [^{14}C]-5-HTP to [^{14}C]-5-HT	mg of protein in the incubation mixture	Enzyme activity in μU[a]	Specific activity in μU[a]/mg of protein
Hepatopancreas	6.0	3.00	219.6	73.2
Eyestalkless heads	4.9	3.82	179.3	46.9
Eyestalks	20.8	2.70	761.3	282.0
Eggs	5.8	11.88	212.3	17.8
Intestine	7.3	3.64	267.2	73.4

The experimental conditions are described in the text. [a] According to the Commission on Enzymes of the International Union of Biochemistry, 1 Unit (IU) of enzyme is defined as that amount of enzyme that catalyzes the production of 1 μMol product per min, while the Units per mg of protein is defined as specific activity of the enzyme.

The enzymic nature of the produced serotonin was tested by measurement of the 5-HTP decarboxylase in the presence of hydroxylamine, a potent inhibitor. 50% inhibition was obtained at 10^{-3} *M* concentration of the inhibitor. As can be seen from the Table, the maximum of the enzyme activity has been found in the eyestalks, the level of which is above that found in rat pineal gland, one of the richest sources of this enzyme[8]. It is interesting that the eyestalkless heads have very little activity. This fact that the eyestalks are the richest source in the heads of decapods, comparable to that occurred in the pineal gland of mammals, led us to consider that serotonin or any of its metabolites may play a role in the colour change of crustaceans. This assumption is in accordance with that found by ÖSTLUND and FAENGE[9], where serotonin causes pigment dispersion in red chromatophores of *Leander adspersus*. In addition the removal of eyestalks causes general darkening in decapod, while injection of eyestalks extract causes blanching[10].

The finding that the eggs have some 5-HTP decarboxylase activity, is in accordance with the conclusions of BUZNIKOW et al.[11] where serotonin, called by them 'embryonic hormone', is involved in the processes of early embryogenesis, both in Protostomia and Deuterostomia. Finally one of us[6] has found changes of 5-HTP decarboxylase activity during the developmental stages of the blow-fly *Calliphora erythrocephala*, which depends on ecdysone titer.

We do not claim to have conclusive evidence for these correlations; more work must be done to clear up these hypotheses.

Zusammenfassung. Die Aktivität der 5-HTP Dekarboxylase in Rohextrakten verschiedener Organe von *Upogebia littoralis* wurde radiochemisch bestimmt. Die grösste Aktivität des Enzyms findet sich in den Stielaugen, während stielaugenlose Köpfe die niedrigste Enzymaktivität zeigen.

V. J. MARMARAS and E. G. FRAGOULIS

Department of General Biology, University of Athens, Athens (Greece), 31 October 1969.

[1] J. H. WELSH and M. MOORHEAD, J. Neurochem. *6*, 146 (1960).
[2] D. M. MAYNARD and J. H. WELSH, J. Physiol., Lond. *149*, 215 (1959).
[3] R. ELOFSSON, T. KAURI, S.-O. NIELSEN and J.-O. STRÖMBERG, Z. Zellforsch. mikrosk. Anat. *74*, 464 (1966).
[4] R. ELOFSSON, T. KAURI, S.-O. NIELSEN and J.-O. STRÖMBERG, Experientia *24*, 1159 (1968).
[5] A detailed description of the assay, properties and specificity of 5-HTP decarboxylase, will be published in a future communication.
[6] V. J. MARMARAS, C. E. SEKERIS and P. KARLSON, Acta biochim., pol. *13*, 305 (1966).
[7] O. H. LOWRY, N. J. ROSEBROUGH, A. LEWIS FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[8] S. H. SNYDER and J. AXELROD, Biochem. Pharmac. *13*, 805 (1964).
[9] E. ÖSTLUND and R. FAENGE, Annls. Sci. nat. Zool. Ser. II, *18*, 325 (1956).
[10] E. FLOREY, *An Introduction to General and Comparative Animal Physiology* (W. B. Saunders Co., Philadelphia 1966).
[11] G. A. BUZNIKOV, I. V. CHUDAKOVA and N. D. ZVEZDINA, J. Embryol. exp. Morph. *12*, 563 (1964).