

planations. Whereas many other workers have had their attention drawn to the multiplicity of ultrastructural features of hepatocytes, in the present study attention was only paid to the detection of centrioles. Although this may in part account for the disparity, other factors are probably involved. Firstly, because of the very large size of the mature hepatocyte and its small nucleo-cytoplasmic ratio, the chances of cutting the centrioles is far less than in most other cell types. Secondly, the liver cell nucleus does not have a discernible hilar region where centrioles are usually found as, for example, in lymphocytes; in the hepatocyte, therefore, attention is not drawn to the area most likely to contain the centrioles. Finally, hepatocytes contain many small vesicular bodies, densely staining glycogen granules, lipid droplets, and lysosomes which tend to obscure the less densely staining centriole (as in Figure 2).

Sufficiently large samples of centrioles in both normal and regenerating liver have been examined to establish the improbability of hepatocytes possessing cilia. Cilia have been detected, however, in capillary endothelial cells (unpublished observations) and in bile duct epithelial cells^{11,12} in the rat liver¹³.

Zusammenfassung. In der normalen und regenerierenden Rattenleber wurden Zentriolen gefunden. In der normalen Leberparenchymzelle erscheinen sie wahrscheinlich als Diplosomen. Häufig werden mehr als 2 Zentriolen in der einzelnen Leberparenchymzelle beobachtet, jedoch gibt es keine Anhaltspunkte für eine zentrioläre Replikation in den normalen Leberparenchymzellen. Teilhepatektomie tritt kurz nach Bildung von Tochterzentriolen auf. Das Vorkommen von Zilien scheint mit den Zentriolen der Leberparenchymzellen nicht verbunden zu sein.

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¹¹ J. W. STEINER, J. S. CARRUTHERS and S. R. KALIFAT, *Expl molec. Path.* 1, 162 (1962).

¹² J. W. GRISHAM, *Proc. Soc. exp. Biol. Med.* 114, 318 (1963).

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Catecholamine-Containing Nerve Fibres in the Hind-Gut of the Crayfish *Astacus astacus* L. (Crustacea, Decapoda)

A number of investigations have been devoted to the intestinal nervous system of *Astacus astacus*¹⁻³. By means of the specific fluorescence method of FALCK and HILLARP⁴ for the cellular localization of certain monoamines, we were able to demonstrate the presence of monoaminergic fibres in this part of the nervous system. In a previous investigation, the central nervous system of *A. astacus* was described according to this method⁵.

There is full agreement in the previous investigations that the innervation of the crayfish intestine derives from 2 main sources; one, the ganglia of the circumesophageal connectives that innervate the anterior part of the intestine; the other, the last abdominal ganglion that innervates the hind-gut. From the last abdominal ganglion in the crayfish, 1 nerve turns dorso-caudal to the hind-gut where it divides into 1 posterior and 2 anterior branches. These give rise to a dense network of fibres, the 'Grundplexus' of ALEXANDROWICZ¹ (p. 403), surrounding the whole hind-gut. Without sharp limits, it continues as a second order plexus, called by ALEXANDROWICZ 'Endplexus', which innervates the muscles. The fibres of this latter plexus are parallel to the muscles in contrast to the 'Grundplexus' where the fibres are randomly distributed. The circular muscle layer is rather thick. The longitudinal muscles, confined to the 6 ridges of the intestine, are concentrated in a few bundles. Their special mode of insertion is described, among others, by ALEXANDROWICZ¹.

There are some statements in the literature with special regard to catecholamines in crustaceans. ÖSTLUND⁶ testing *Daphnia pulex* and *Crangon crangon* and v. EULER⁷ working on *Carcinus maenas* could not demonstrate any appreciable amounts of adrenaline and noradrenaline in extracts of whole animals. Recently KERKUT, SEDDEN and WALKER⁸ reported the presence of dopamine in the thoracic nerve mass of *C. maenas* in a concentration of 7.3 µg/g. They found no adrenaline or noradrenaline.

FLOREY⁹ found that adrenaline and noradrenaline were active on the hind-gut of the crayfish *Cambarus clarkii* increasing contraction height. However, he concluded that this effect was not specific and suggested that the compound active on the gut was of a structure similar to adrenaline or noradrenaline.

In the present experiment fibres emitting a green fluorescence, characteristic for catecholamines, were observed in both plexa mentioned above, whereas no fibres emitting a yellow fluorescence, which would indicate the presence of 5-hydroxytryptamine, were found. The green fluorescent fibres seem to constitute only a minor part of the plexa, which according to ALEXANDROWICZ contain a large amount of fibres. The fluorescent fibres are found among both the circular and the longitudinal muscle fibres. In the circular muscle layer, they are more abundant in the outer part, the 'Grundplexus', where they are thicker and of a less typical beaded appearance than those of the 'Endplexus'. In the latter, they appear as thin threads with numerous varicosities (Figure). Among the muscle fibres, sparsely distributed bulb-shaped structures are also found. They contain several green fluorescent granules. It is not known whether these represent terminations of the varicose fibres. Besides the green fluorescent

¹ J. S. ALEXANDROWICZ, *Jena. Z. Naturw.* 45, 395 (1909).

² E. JANISCH, *Z. wiss. Zool.* 121, 1 (1923).

³ J. ORLOV, *Z. mikrosk.-anat. Forsch.* 4, 101 (1926).

⁴ B. FALCK and C. OWMAN, *Acta Univ. lund.*, Sect. 2, 7, 1 (1965).

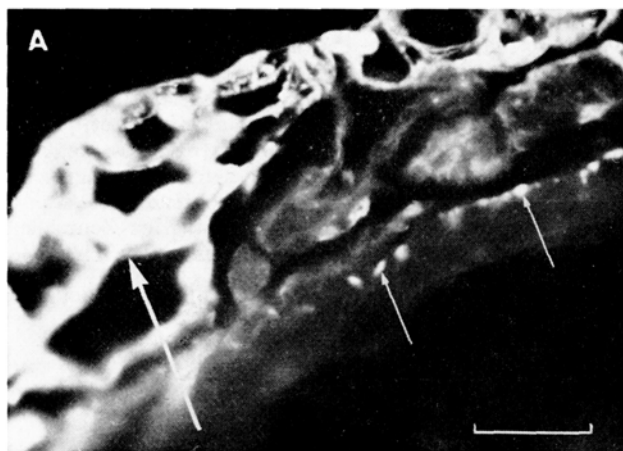
⁵ R. ELOFSSON, T. KAURI, S.-O. NIELSEN and J.-O. STRÖMBERG, *Z. Zellforsch. mikrosk. Anat.* 74, 464 (1966).

⁶ E. ÖSTLUND, *Acta physiol. scand.* 31, Suppl. 112, 1 (1954).

⁷ U. S. VON EULER, *Nature* 190, 170 (1961).

⁸ G. A. KERKUT, C. B. SEDDEN and R. J. WALKER, *Comp. Biochem. Physiol.* 18, 921 (1966).

⁹ E. FLOREY, *Z. vergl. Physiol.* 36, 1 (1954).



Hind-gut of *Astacus astacus* (L.) Sections of the gut, showing green varicose fibres (varicosities indicated by small arrows). Length of index line, 25 μ . (A) Transverse section. The big arrow indicates the



peripheral, strongly fluorescent connective tissue. Circular musculature is seen below the connective tissue layer. (B) Longitudinal section through the longitudinal musculature.

nerve fibres mentioned above, no other fluorescent nerve fibres could be detected. Thus the bipolar sensory neurons have not been found to react to this method.

Three animals were treated with reserpine (Serpasil, CIBA, 19 mg/kg body weight – exoskeleton excluded) 24 h before killing. No specific fibre fluorescence was observed in these specimens. This supports the presumption that the nervous fluorescence derives from a monoamine.

A moderate green autofluorescence and numerous autofluorescent brownish-yellow spots were present in all preparations. A strong formaldehyde-induced fluorescence, confined to the loose connective tissue surrounding the gut, was observed in the specimens treated according to the method of FALCK and HILLARP (Figure A). This fluorescence was not affected by pretreatment with reserpine. A similar fluorescence reaction has been found previously in the epineurium around the CNS⁵.

Regarding the monoamines that can be demonstrated with this method, apart from 5-hydroxytryptamine discussed above, the green fluorescence can be caused by the presence of adrenaline, noradrenaline, and dopamine either singly or in combinations. Prolonged treatment of the tissues by formaldehyde gas in order to demonstrate adrenaline did not give an increased fluorescence intensity or increase of the number of fluorescent structures, thus indicating the absence of adrenaline.

In attempting to differentiate between noradrenaline and dopamine, excitation spectra were recorded from the varicosities of the 'Endplexus' using microspectrofluorometry¹⁰. Preliminary results with this method give evidence of the presence of noradrenaline but do not exclude the possibility of dopamine also being present¹¹.

Zusammenfassung. Katecholamin enthaltende Nervenfasern im Enddarm von *Astacus astacus* (L.) werden mit Hilfe von FALCKS und HILLARPS spezifischer Fluoreszenzmethode gezeigt. Die Fasern, die eine grüne Fluoreszenz abgeben, wurden in Nervenplexen der zirkulären und der längsgehenden Muskelschichten des Darmes beobachtet. Es wird angenommen, dass diese Fluoreszenz durch Noradrenalin zustande kommt.

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Zoological Institute, 22362 Lund (Sweden), 22 July 1968.

¹⁰ A. BJÖRKLUND, B. EHINGER and B. FALCK, J. Histochem. Cytochem. 16, 263 (1968).

¹¹ The research reported in this document has been sponsored by the Swedish Natural Science Research Council under grant No. 99-35.

Les chromosomes de *Leggadina hermannsburgensis hermannsburgensis* Waite et le problème des Muridae australiens

On sait que, à l'exception du Chien Dingo probablement introduit par l'Homme à une époque relativement récente, le continent australien, à l'arrivée des Blancs, ne possédait, en fait de Mammifères placentaires, que des Chiroptères et des Rongeurs, parmi ceux-ci 13 genres de Muridae contenant 19 groupes d'espèces¹. En adjoignant à l'Australie, la Nouvelle-Guinée, Céram et quelques autres îles, on arrive à un total de 25 genres avec 245 formes nommées. L'Australie se trouve coupée de toute liaison avec l'Asie dès le Crétacé supérieur², soit depuis 50 millions d'années³, cette connexion ancienne n'apparaissant même

pas dans les cartes de TERMIER⁴. JOLEAUD⁵ estime que l'isolement de l'Australie date du Crétacé ancien mais que, même après les temps mésozoïques, elle fut réunie à l'Asie à diverses reprises par la Nouvelle-Guinée, les îles de la Sonde et les Philippines. Les Hydromyinae seraient arrivés à l'Oligocène alors que les Murinae auraient utilisé une jonction asiatico-australienne via les Philippines et la Malaisie, au Pliocène. Rappelons que si des Muridés bien caractérisés ne sont pas connus avant le Pliocène et que si LAVOCAT⁶ leur attribue une origine sud-asiatique, «SIMPSON (1945) fait remonter leur origine à la fin du