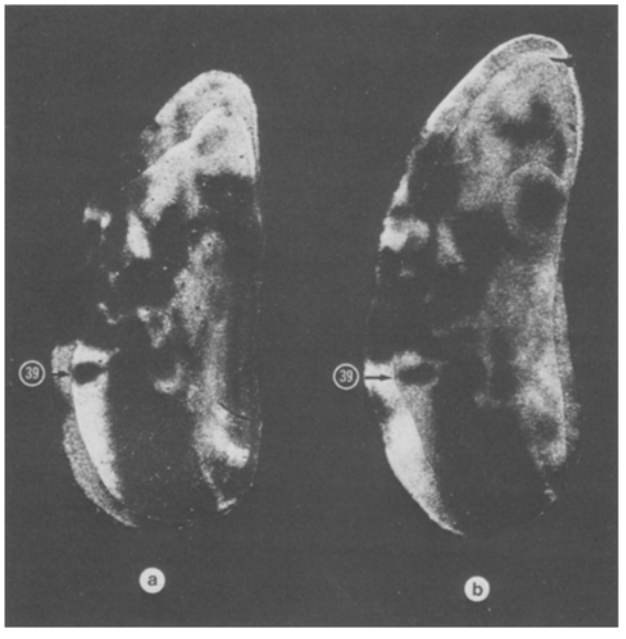
ether anestesia. In all experiments as well as in the controls the animals were killed by cardiac perfusion with 5% formalin under ether anestesia 3 days after callosotomy. Series of frozen sections were prepared in horizontal planes and stained according to a new and reliable method that impregnates lysosomes and degenerating terminals[9,10].

In all experiments the patterns of the callosal connections was not different from those characteristic of normal animals[11-13] (fig.).

The packing density of the impregnated particles was measured by a television image analysis system using an electronic device for detecting small black spots (Quantimet 720). From each of the 6-OHDA, the propranolol, and the control groups 2 animals were evaluated. Area 39 (according to Krieg[14]) was selected for these measurements because of its small size and the constant occupation of its borders by callosal connections in the rat[11]. We observed only small deviations from the controls. According to the Mann-Withney U-test the decrease in the density found following the 6-OHDA or propranolol treatments was not significant. The data are sufficient to exclude the possibility that the callosal connections were augmented. Hence, it can be excluded that in the neocortex of rats the inhibition of the NA-ergic transmission increases the synaptogenetic potential of the callosal fibers during the normal development. This finding is in contrast to what has been shown for the ventral hippocampal commissure after entorhinal lesion[3]. The discrepancy may be due to a different effect of NA on normal and reactive synaptogenesis or to NA acting differently in various regions of the CNS. In both cases the morphogenetic effect of NA might not be determined by a general effect at the single cell level. The specifity of NA effects could rather depend on the local structure of neuronal circuits.



Pattern of degeneration in the supragranular layers of the hemisphere contralateral to callosotomy, reconstructed from horizontal sections (darkfield illumination). *a* Control animal without any antinoradrenergic treatment, *b* animal treated with propranolol between days 14 and 20 p.n. The position of area 39 is indicated by arrows at the left border of the micrographs. Minor differences in the callosal pattern along the medial border of area 39, the anterior border of area 17, and in the frontal cortex are due to interindividual variations. The differentiation of the principal pattern of interhemispheric connections is not changed by anti-NA-ergic treatment during the development of callosal afferents in the supragranular layeres (a and b).

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2 Lynch, G., Rose, G., Gall, Ch., and Cotman, C.W., in: Golgi centennical symposium, p.305. Ed M. Santini. Raven Press, New York 1975.
3 Amaral, D.G., Avendano, C.A., and Cowan, W.M., J. comp. Neurol. *194* (1980) 171.
4 Cotman, C.W., and Lynch, G.S., in: Neural recognition, p.69. Ed. S. Barondes. Plenum Press, New York 1976.
5 Kasamatsu, T., and Pettigrew, J.D., J. comp. Neurol. *185* (1979) 139 and 163.
6 Sachs, Ch., J. Neurochem. *20* (1973) 1753.
7 Wiese, U.H., The development of the callosal system in the albino rat, in preparation.
8 Sternberg, D.B., and Gold, P.E., Behav. neural Biol. *29* (1980) 289.
9 Gallyas, G., Zaborzky, L., and Wolff, J.R., Stain Technol. *55* (1980) 281.
10 Gallyas, F., Wolff, J.R., Böttcher, H., and Zaborzky, L., Stain Technol. *55* (1980) 299.
11 Wolff, J.R., and Zaborzky, L., in: Structure and function of cerebral commissures, p.147. Eds J. Steel Russel, W.M. van Hof, and G. Berlucchi. The MacMillan Press Ltd, London and Basingstoke 1979.
11a Zaborzky, L., and Wolff, J.R., Anat. Embryol., submitted.
12 Wise, S.P., and Jones, E.G., J. comp. Neurol. *168* (1976) 313.
13 Ivy, G.O., and Killackey, H.P., J. comp. Neurol. *195* (1981) 367.
14 Krieg, W.J.S., Connections of the cerebral cortex. Brain Books, Evanston, Ill. 1963.

Axoneme patterns of spermatozoa of Asian horseshoe crabs[1]

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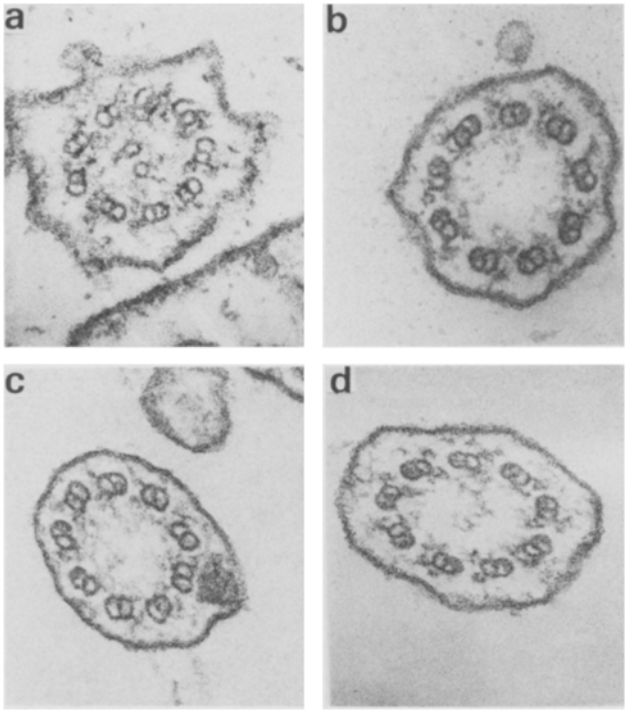
Summary. Ultrastructural studies of flagella of spermatozoa of horseshoe crabs revealed that three Asian species *(Tachypleus tridentatus, T. gigas* and *Carcinoscorpius rotundicauda)* had a 9+0 axoneme pattern that was different from the usual 9+2 pattern of *Limulus polyphemus.* This difference is consistent with the phylogeny of horseshoe crabs.

Numerous studies on the spermatozoa of Arthropoda have shown wide structural diversity in this phylum[3]. The spermatozoa of the American horseshoe crab, *Limulus polyphemus,* are reported to have a unique acrosomal filament and the usual 9+2 pattern of the flagellum[4-8]. This paper is the first report on the ultrastructure of the sperm flagella of 3 Asian horseshoe crabs. Japanese horseshoe crabs, *Tachypleus tridentatus,* were collected in the vicinity of Imari,

Japan, and Southeast Asian horseshoe crabs, *Tachypleus gigas* and *Carcinoscorpius rotundicauda,* were kindly provided by Dr Smarn Srithunya (Zoological Museum, Srinakharinwirot University, Thailand). American horseshoe crabs, *L. polyphemus,* which were reexamined, were supplied from the Department of Marine Resources, Marine Biological Laboratory, Woods Hole, Mass., USA. The spermiducts of the 4 species were fixed in 2% glutaraldehyde, buffered with 0.2 M sodium cacodylate (pH 7.4) and containing 7% sucrose, for 1 h, washed with the buffer for 3 h and postfixed in 2% osmic acid in the same buffer for 1 h. Then the specimens were dehydrated in an ethanol series and embedded in Spurr Resin. Ultrathin sections were cut with glass knives, stained with uranyl acetate and lead nitrate, and observed in a JEOL's JEM 100-C electron microscope. In contrast with the axoneme of *L. polyphemus* (fig., a), which has the 9+2 pattern, the 3 Asian species had a 9+0 pattern without central tubules (fig., b, c, d). These spermatozoa were motile. Costello suggested that 9+2 spermatozoa have a planar motion, while 9+1 or 9+0 spermatozoa have a helical one[9,10]. We are studying the details of the motion of the spermatozoa, the ultrastructure of their other organellae and the species specific interaction of spermatozoa with eggs. Baccetti reported that among arthropods only *Limulus* has the basic aquatic sperm whose flagella have the usual 9+2 pattern, without other accessory fibrils, and he proposed a monophyletic origin of arthropods, starting from a *Limulus*-like aquatic ancestor[3]. On the basis of comparative studies on the sperm, he proposed various common evolutionary pathways in Arthropoda with similar steps, whose first step is the acquisition of unusual axoneme patterns[3]. According to his hypothesis and our observations, Asian horseshoe crabs appear to have evolved further than *L. polyphemus.* This idea seems to be consistent with data on fossils suggesting that Limulinae and Tachypleinae branched off in the Jurassic period[11], and also consistent with the results of hybridization experiments[12], the constitution of hemocyanin monomers[13] and phylogenetic analyses based on amino acid sequences of coagulogens[14].



Axoneme patterns of horseshoe crabs. *a Limulus polyphemus* (9+2); *b Carcinoscorpius rotundicauda* (9+0); *c Tachypleus tridentatus* (9+0); *d Tachypleus gigas* (9+0). × 112,000.

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3 Baccetti, B., in: Arthropod phylogeny, p. 609. Ed. A.P. Gupta. Von Nostrand Reinhold Co., New York 1979.
4 Philpott, D.E., and Shaw, W.N., Biol. Bull. *117* (1959) 423.
5 Andre, J., Annls Fac. Sci. *26* (1963) 27.
6 Shoger, R.L., and Brown, G.G., J. submicr. Cytol. *2* (1970) 167.
7 Brown, G.G., and Humphreys, W.J., J. Cell Biol. *51* (1971) 904.
8 Fahrenbach, W.H., J. Morph. *140* (1973) 31.
9 Costello, D.P., Biol. Bull. *145* (1973) 279.
10 Costello, D.P., Biol. Bull. *145* (1973) 292.
11 Shuster, C.N., in: Encyclopedia of science and technology, vol. 14, p. 643. McGraw-Hill Book Co., London 1977.
12 Sekiguchi, K., and Sugita, H., Evolution *34* (1980) 712.
13 Sugita, H., and Sekiguchi, K., in: Invertebrate oxygen-binding proteins: structure, active site and function, p. 247. Eds J. Lamy and J. Lamy. Marcel Dekker, New York 1981.
14 Shishikura, F., and Sekiguchi, K., Zool. Mag. *88* (1979) 667.

Diphasic action of 2,2-diphenylpropionic acid N,N-diethylaminoethyl ester hydrochloride on hepatic drug metabolism in the mouse

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Summary. 2,2-Diphenylpropionic acid N,N-diethylaminoethyl ester hydrochloride, 30 min after a single i.p. dose, inhibits the microsomal drug-metabolizing enzymes of the mouse liver and prolongs the hexobarbital sleeping time; 24 h after the administration, it induces hepatic microsomal drug-metabolizing enzymes and shortens hexobarbital sleeping time.

Some organic compounds such as piperonyl butoxide[1], malonic acid derivatives[2], 2-hydroxy-2-ethylbutyryl N,N-diethylamide[3], hexobarbital[4], and diazepam[5], act both as inhibitors and inducers of hepatic drug metabolism. The induction of drug metabolism which occurs after initial drug biotransformation inhibition, is an adaptive process of the liver in order to overcome enzyme inhibition by these substances[6,7]. This paper is concerned with the same diphasic action on drug metabolism exerted by the basic ester, 2,2-diphenylpropionic acid N,N-diethylaminoethyl