was used for fluorimetric measurements. Readings were made at activation wave lengths of 380 and 430 nm and at fluorescence wave lengths of 490 and 540 nm. The mean values ± SEM are given in ng/g of tissue.

Results. In the fetuses of 30 days, the brain norepinephrine level is low, only 51 ± 4.9 ng/g. No changes occur the next day, the last one before birth (52 ± 9.7 ng/g), as can be seen in the Figure.

After parturition, within the 1st h, the newborn rabbit brain content falls to 34 ± 9.9 ng/g norepinephrine, about 37% less than that of the 31st day. Later on, within 2 to 4 h, the amount of norephinephrine returns to the prenatal level (52 ± 5.6 ng/g) and remains practically the same for 8 to 12 h (51 ± 2.4 ng/g) (see Figure).

Discussion. In the rat fetus, the enzymes involved in the biosynthesis of catecholamines are present in the brain at 15 days of gestation[8,9]; at this age, the norepinephrine is at only 2% of the adult level, whereas the biosynthetic enzymes have specific activities of about 10% of those in the adult brain[10]. At birth and thereafter, the biosynthetic enzymes and their products, dopamine and norepinephrine, increase in a parallel fashion[9,11]. Our results show that in the rabbit fetus on the 30th and 31st day of gestation, norepinephrine is present and the level remains unchanged, as is the case within 2 to 4 and 8 to 12 h after parturition. On the other hand, during the 1st h following parturition, a decrease of norepinephrine can be seen and the variability of the individual values is larger than in the other stages. Within 2 to 4 h, the amount of norepinephrine rises to the level measured in the fetuses, and remains the same for 8 to 12 h. Similar modifications of the amount of catecholamines have been observed in the newborn rabbit, in various tissues. For instance, during the first few hours following parturition, one can observe a decrease of the amount of catecholamines in the adrenals[12,13] and increase in the plasma[13,14] and in the heart[15]. These rapid and transitory variations of the amounts of catecholamines found in the newborn rabbit thus seem to be linked to birth. The fall of the norepinephrine level in the brain can be related to the fetal suffering special conditions during parturition, especially the hypoxia which occurs[16]. It has been established that in the adult cat, the asphyxia produced by rebreathing can decrease the norepinephrine level in the hypothalamus in less than 2 h[17]; in the adult rat, the hypoxia decreases in 1 h the norepinephrine in the brain[18]. In the fetus, asphyxia also introduces a decrease of the level of catecholamines in human[19], lamb[20], calf[21] and foal[22] adrenals and in human[19] and rabbit[23] paraganglia as well as in human[24] extra-adrenal tissue. In the adult rat, other severe stresses, such as prolonged muscular effort[25] and exposure to cold[26–28], are also known to reduce the endogenous level of catecholamines in tissues. Consequently, the spontaneous decrease of norepinephrine in the brain which we have found in the newborn rabbit can also be explained by stress conditions to which the animals are exposed during parturition. As has been suggested[29], norepinephrine, by its vasomotor action, could contribute to a better distribution of blood to the brain, thus increasing the brain's oxygen stock and facilitating the defense of the newborn animal against asphyxia. Therefore, this brain norepinephrine liberation during parturition could have an important role in a most critical period of the mammal's life[30].

Summary. In the first hour after parturition, the newborn rabbit brain norepinephrine content is about 37% less than that of the fetus of 30th or 31st day. Later on, within 2 to 4 h, the norepinephrine level returns to the prenatal value and remains unchanged between 8 to 12 h. This transitory fall of the brain norepinephrine seems to be linked to the stress conditions which occur during parturition.

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Comparative Study of the Electrical and Mechanical Behaviour of an Intact, Semi-Intact and Isolated Gastropode (*Helix pomatia*) Smooth Muscle Preparation

Under appropriate stimulation conditions, the isolated penis retractor muscle (PRM) of *Helix pomatia* L., a gastropode smooth muscle, can be made to perform a phasic contraction and a prolonged contraction known as 'catch' (Wabnitz[1,2]). Whether the two distinct types of contraction play a part in the normal behaviour of the penis retractor muscle in the intact animal is unknown.

The aim of the present experiments is to compare the normal electrical and mechanical behaviour of the intact penis retractor muscle-nerve-brain preparation with the properties of the muscle at different stages of surgical

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isolation in order to clarify the question to what extent the PRM is controlled by the central nervous system, or by peripheral mechanisms, and whether the pattern of muscle activity initiated by artificial stimulation is comparable with the muscle activity in vivo. In the following study 3 different types of preparations of the PRM were used: 1. the intact PRM-nerve-brain preparation (Figure 1), 2. the semi-intact preparation consisting of the PRM and the peripheral nerve plexus (Figure 2) and 3. the isolated PRM preparation without any connection to identified peripheral neuronal cell clusters (Figure 2).

For up to 1 h after dissection, an asynchronous muscle activity was recorded in all 3 types of preparations indicated by small, irregular spike amplitudes and the relative high frequency of the spike discharge (Figure 3). After this time, however, the intact and the semi-intact preparation became more and more synchronized and a

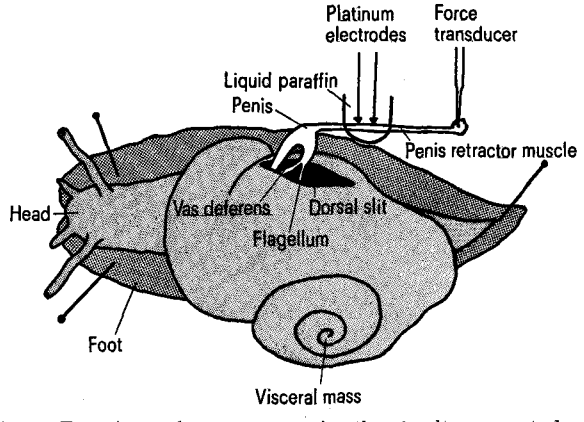

Fig. 1. Experimental arrangement for the simultaneous study of the mechanical and electrical activity of the intact penis retractor muscle-nerve-brain preparation of *Helix pomatia*. The snail was immobilized by pinning the foot to the waxfloor of a chamber containing oxygenated ringer solution at 21 °C. After removal of the shell one end of the PRM was exposed through a dorsal slit in the mantle tissue and connected to a force transducer for auxotonic tension measurements. The electrical activity was recorded extracellularly by 2 platinum electrodes spaced 4 mm apart and isolated by liquid paraffin.

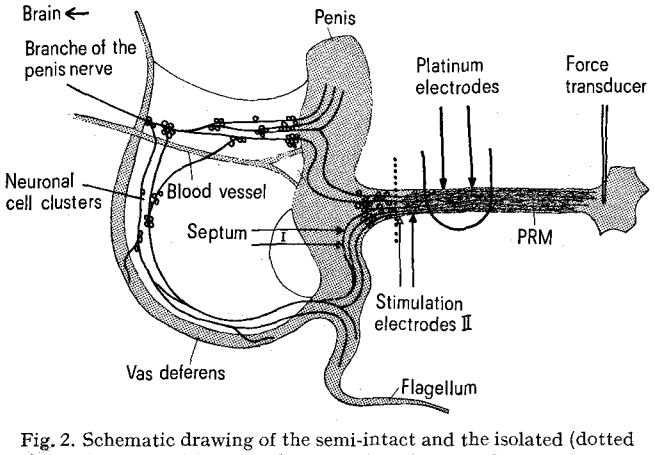

Fig. 2. Schematic drawing of the semi-intact and the isolated (dotted line indicating incision) PRM preparation showing the arrangement for stimulation and external recording. Some branches of the penis nerve traversing the septum between the vas deferens and the penis run to supply the penis retractor muscle. Clusters of nerve cells are scattered around the nerve branches. Note especially the clusters at the base of the PRM.

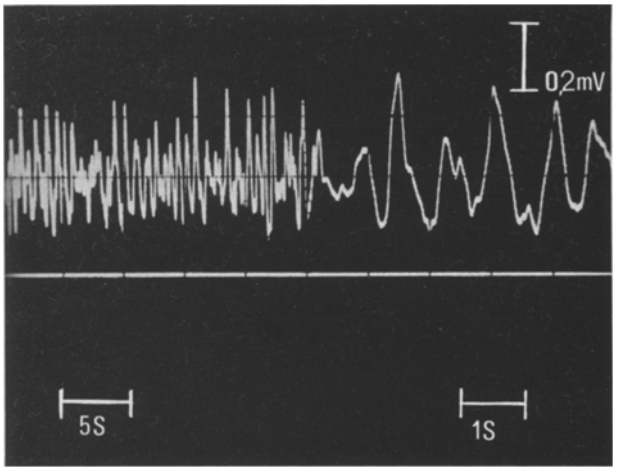

Fig. 3. Spontaneous electrical activity (upper trace) and auxotonic tension development (lower trace) of the penis retractor muscle recorded 40 min after dissection (low speed on the left and higher speed on the right).

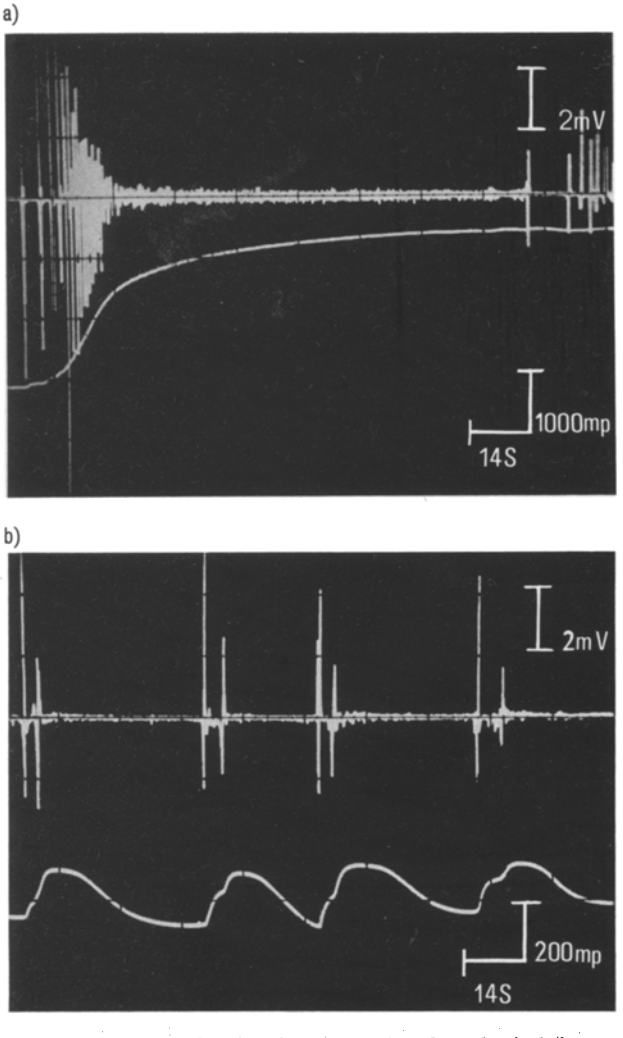

Fig. 4. Spontaneous electrical (upper trace) and mechanical (lower trace) activity of the intact penis retractor muscle-nerve-brain preparation (see Figure 1) recorded 120 min after dissection. a) Long duration burst of spike activity and prolonged contraction. b) Short duration burst of spike activity and phasic contraction.

specific pattern of spontaneous muscle activity is stabilized.

The experiment illustrated in Figures 4a and 4b shows a typical example for the electrical and mechanical activity of the intact muscle-nerve-brain preparation. There are principally 2 types of electrical muscle activity, a) a long duration burst of spike activity lasting up to 30 min and b) a short duration burst of spike activity lasting less than 60 sec. The long duration bursts were repeated at intervals of 10 to 30 min. The interval duration increased with time. During the long duration bursts, a drop in the muscle cell synchronization occurred, accompanied by a strong prolonged contraction. During tension development and the first part of the plateau phase of contraction, the spike amplitude and the interspike interval decreased, followed by a period of increasing amplitude of superimposed spikes indicating an increase of the electrical muscle cell synchronization. The short duration bursts (Figure 4b), which were observed during relaxation and after complete relaxation of the strong prolonged contraction, showed interburst-intervals of 20 sec up to several min and were associated with smaller phasic contractions. Recordings from the same preparation, under identical conditions but 8 h after dissection, showed a changed pattern of burst activity. The short duration bursts had almost disappeared, while burst duration and the tension development correlated to the long duration bursts increased and the burst repetition frequency decreased.

In contrast to these observations, it was found that in the semi-intact penis retractor muscle preparation (see Figure 2) long duration bursts did not occur. The short duration bursts were unaffected by the surgical treatment indicating that the long duration burst activity originated from the central nervous system while the remaining short duration activity was initiated by regularly firing peripheral structures located possibly at the base of the PRM (Figure 2) which is found to contain a group of neurons (EBERHARDT and WABNITZ, in preparation). Figure 5 shows a representative example of the pattern of electrical activity in the semi-intact preparation, which was maintained for hours with only little changes in the spike amplitudes, spike repetition frequency, burst duration and interburst intervals.

In a third group of experiments using the isolated penis retractor muscle preparation (Figure 2), it was found that 1 h after dissection the spontaneous activity was completely abolished. Therefore, myogenic activity seems not to be the underlying mechanism for the spontaneous activity in the PRM.

In the following experiments, the electrical and mechanical responses of the semi-intact nerve-muscle preparation and the isolated muscle preparation (Fig. 2) to indirect and direct stimulation via bipolar platinum electrodes were examined in order to determine the origin of the spontaneous activity. Results from typical experiments are shown in Figures 6a and 6b. One pair of stimuli (square pulses, 0.4 msec., 3V, 1Hz, 2s) applied to the PRM-nerves (electrodes in position I, see Figure 2) produced a long duration burst of spike activity accompanied by a prolonged contraction (Figure 6a).

When the isolated PRM-preparation was directly stimulated with a single pulse above threshold strength (electrodes in position II, see Figure 2), a single spike was elicited and a twitch-like mechanical response was produced (Figure 6b). When the direct stimulation was applied repetively at frequencies equal to or greater than 0.3/s, additional spikes were elicited and a summation of the mechanical responses was observed.

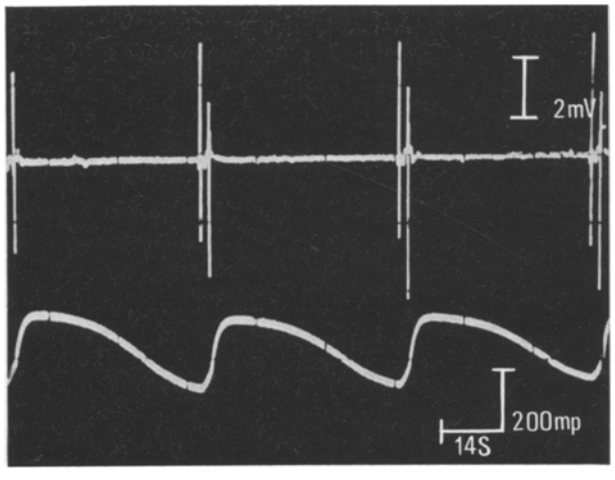

Fig. 5. Electrical (upper trace) and mechanical (lower trace) activity of the semi-intact penis retractor muscle (see Figure 2) recorded 120 min after dissection.

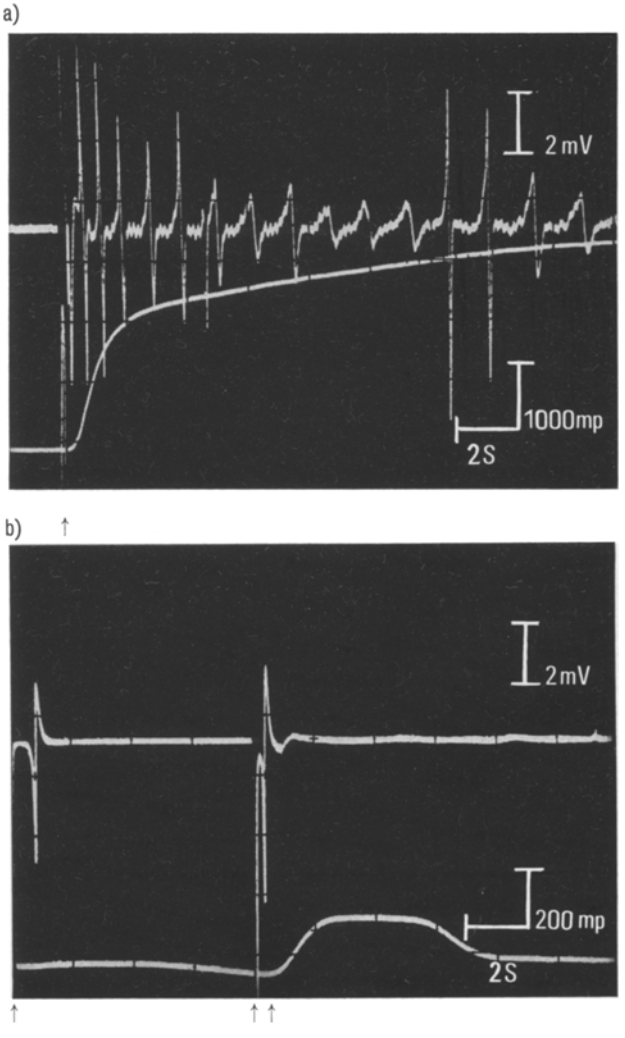

Fig. 6. Electrical (upper trace) and mechanical (lower trace) responses of the penis retractor muscle to stimulation (arrangement see Figure 2): a) Indirect stimulation, electrodes in position I (↑, 0.4 msec, 3V, 1Hz, 2s). b) Direct stimulation, electrodes in position II (↑: 0.2 msec, 3V; ↑↑, 0.4 msec, 3V).

The results described above have shown that 2 types of spontaneously occuring muscle activity are present in the intact penis retractor muscle-nerve-brain preparation resembling those of the stimulated semi-intact respectively the isolated PRM preparation. On the basis of the foregoing experiments, one can conclude that the muscle responses to stimulation reflect normal functioning in the penis retractor muscle.

Beyond that the results demonstrate that the spontaneous muscle activity seems not to be a myogenic mechanism. The functional differences between the intact and the semi-intact preparation suggest that in the absence of obvious sensory input, central neuronal structures are involved in causing the long duration bursts associated with the strong prolonged contraction, while the spontaneously occurring phasic muscle activity seemed to be regulated by peripheral neuronal structures. The latter appears to be due to the repetive activity of nervous elements at the base of the PRM.

Peripherally mediated responses are common in external effector systems of molluscs (KANDEL[3], KUPFERMANN[4], PERETZ[5], WILLOWS[6]) for instance in *Helix pomatia* tactile stimuli applied to the penis without connection to the central nervous system (Figure 2) evokes the contraction of the PRM (WABNITZ, in preparation), indicating a simple peripheral reflexive behaviour.

The experiments described in the second part of this study, showing that the type of contraction produced by stimulation depends upon the position of the stimulation electrodes, corroborate this. The muscle activity elicited by stimulation the PRM-nerve (electrodes in position I, see Figure 2) resembles the spontaneously occurring long duration burst of the intact preparation indicating a modulatory influence of the CNS on the spontaneously active peripheral neurons or a direct control of the muscle by the central nervous system. The synchronous excitory electrical activity of the muscle cells after direct stimulation is similar to the spontaneously occurring muscle response of the semi-intact preparation.

Zusammenfassung. Im intakten Penisretraktormuskel-Nerv-Gehirn-Präparat von *Helix pomatia* L. treten sowohl tonische als auch phasische Muselkontraktionen auf. Die phasische Kontraktion scheint von peripheren Neuronen gesteuert zu werden, während für die Steuerung der tonischen Kontraktion zentrale Strukturen eine Rolle spielen.

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Development of Osmolarity in Blood Plasma and Cerebrospinal Fluid of Chick Embryos

Physical and chemical properties of cerebrospinal fluid (CSF) in chick embryos perform very important changes during the embryonic development. The differences in chemical composition of embryonic blood plasma[1] and CSF[2,3] give some evidence for the maturation of blood-brain barrier. One of the important factors of the CSF-plasma relationship may be the osmotic pressure of both fluids, which was the subject of our present developmental study.

Material and methods. The chick embryos of white Leghorns at the ages from day 11 of incubation to the first posthatching day were used. The osmolar concentration was read out from Knauer semi-microosmometer (type M, model 1970, West Berlin), which requires 150 µl of fluid for 1 determination. Therefore it was necessary to collect for 1 determination the CSF and blood samples from several embryos in dependence on the embryonic age (Table). The CSF was withdrawn from the apical part of the IVth ventricle[4]. The blood was taken till day 15 from the umbilical artery and from the heart in older embryos and in 1-day-old chicks. The glass heparinized capillaries with sharpened tip were used for collection of CSF and blood. The same capillaries were used for centrifugation (2,000 rpm, 20 min) of the CSF and blood samples.

Results. The results are summarized in the Table. The osmolarity of both plasma and CSF increased from day 11 of incubation till the first posthatching day. The osmolarity of blood plasma increased by 35.4 mosm/l

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Age of incubation (days)	No. of embryos	No. of determinations	Osmolarity (mosml)		P/CSF ratio	P-CSF difference (mosm/l)
			CSF	Plasma		
11	35	6	245.0 ± 0.84[a]	248.1 ± 1.27[a]	1.013	3.1
13	30	6	248.2 ± 1.17	251.1 ± 1.34	1.012	2.9
15	30	6	252.6 ± 2.17	257.0 ± 2.03	1.017	4.4
17	25	5	261.6 ± 2.22	270.2 ± 2.12	1.033	8.6
19	28	6	270.0 ± 1.96	280.0 ± 2.77	1.037	10.0
21	18	6	274.8 ± 1.97	280.8 ± 1.88	1.023	6.0
day 1 posthatch.	13	6	276.9 ± 1.93	283.5 ± 1.25	1.023	6.6

[a] $M \pm$ S.E.