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.

(= 14,2%) and CSF osmolarity by 31.6 mosm/l (= 13%).

The development of osmolarity was very mild between day 11 and 13: by 3.2 mosm/l for CSF and by 3.0 mosm/l for blood plasma. The increase on day 15 was only significant ($p < 0.01$): 7.6 mosm/l for CSF, 8.9 mosm/l for blood plasma. A further significant elevation occurred between day 15 and 19 of incubation. The osmolarity increased in CSF by 17.4 mosm/l (= 6.8%) and by 23.0 mosm/l (= 8.9%) in blood plasma. The final developmental increase till the first posthatching day was more moderate and non-significant: in CSF by 6.9 mosm/l (= 2.5%), in blood plasma by 2.5 mosm/l (= 1.2%).

The osmolarity of both these fluids was identical till day 15 of incubation. The difference of 2.9–4.4 mosm/l and the plasma/CSF ratio of 1.012–1.017 were not significant. The osmolarity of blood plasma was regularly higher than the osmolarity of CSF only from day 17 of incubation. On day 17 the plasma-CSF difference increased to 8.6 mosm/l and the plasma/CSF ratio to 1.033. The maximum difference (10.0 mosm/l) and plasma/CSF ratio (1.037) was reached on day 19 of incubation. The values of both these parameters slightly decreased before and after hatching at the steady hyperosmolarity of blood plasma in comparison with the CSF.

*Discussion.* The direct measurements of plasma and CSF osmolarity did not confirm our previous theoretical presumption on the hyperosmolarity of CSF, which was calculated from the concentration of some ions, glucose and total proteins[3].

The developmental increase of the CSF and plasma osmolarity was parallel till day 19 of incubation. The increase of plasma osmolarity before and after hatching was smaller than in the CSF, which resulted in the anomaly in comparison with the development before day 19 of incubation. The development of chemical composition of the CSF and blood plasma[2,3], and especially the above-mentioned fact, showed that there probably was a barrier developing after day 15 of incubation, which ensured the difference between the concentration of osmotically active components in the CSF and blood plasma and which was responsible for a different development of osmolarity in both fluids, especially during the last days of incubation and after hatching. Consequently, the osmotic gradient from the blood plasma into the CSF must be taken into account since day 15 of incubation among the factors influencing the CSF formation and regulation of the CSF volume in the cerebral ventricular system[5] during this stage of the ontogenetic development.

*Résumé.* La pression osmotique du plasma sanguin et du fluide cérébrospinal (CSF) a été mesurée chez les embryons de poulet entre le 11e jour d'incubation et l'éclosion, et chez les poussins d'un jour. La pression osmotique augmente de 248,1 à 283,5 mosm/l dans le plasma sanguin et de 245,0 à 276,9 mosm/l dans le CSF. Dès le 11e jour du développement le plasma sanguin est hyperosmotique par rapport au CSF, ce qui prouve l'établissement d'une barrière osmotique entre le plasma sanguin et le CSF.

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## Interaction Between Sugars and Amino Acids in Intestinal Absorption by Rat, in vivo

Interaction between sugars and amino acids at intestinal level has received much attention in recent years. Cividanes et al.[1] showed that glucose and glycine absorption by rat intestine in vivo exhibit a mutual inhibition, but Bingham et al.[2] did not find any effect on amino acid absorption when glucose was present. More recently, Cooke[3] reported interaction between these substrates in man, and Bolufer et al.[4] have referred to the effect of leucine, glycine and arginine on glucose and galactose absorption by rat intestine, always on in vivo experiments. In the present paper, the mutual interaction between glucose, galactose and leucine absorption by the rat small intestine is studied in vivo.

*Methods.* Wistar rats of either sex of 150–200 g body weight were used. The animals were starved for 24 h

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L-leucine absorption by small intestine of rat in vivo at various initial concentrations

| L-leucine (m*M*) | Absorption (μmol leucine/cm intestine) | | | | |
|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 5th |
| 1[a] (4) | 0.21 ± 0.02 | 0.20 ± 0.02 | 0.22 ± 0.01 | 0.21 ± 0.01 | 0.20 ± 0.01 |
| 4[a] (4) | 0.80 ± 0.01 | 0.78 ± 0.12 | 0.78 ± 0.14 | 0.84 ± 0.10 | 0.81 ± 0.11 |
| 20[a] (5) | 2.91 ± 0.13 | 2.83 ± 0.08 | 2.87 ± 0.08 | 2.78 ± 0.12 | 2.80 ± 0.09 |
| 1 (6) | 0.07 ± 0.00 | 0.07 ± 0.01 | 0.07 ± 0.01 | 0.07 ± 0.00 | 0.08 ± 0.01 |
| 4 (4) | 0.43 ± 0.05 | 0.41 ± 0.04 | 0.40 ± 0.04 | 0.41 ± 0.06 | 0.42 ± 0.05 |
| 20 (6) | 1.55 ± 0.10 | 1.50 ± 0.08 | 1.53 ± 0.09 | 1.57 ± 0.12 | 1.51 ± 0.07 |

The solution was recycled by peristaltic pumping or left in the intestinal loop. Each absorption period was of 5 min. Number of animals is given in brackets. The data are the mean ± SE. [a] With peristaltic pumping of 10 ml of solution with the appropriate added substrate through a closed circuit at a 13.54 ml/min rate.