Whole hydras were pretreated in 10 µg/ml AD separately for 24 and 48 h and subsequently were amputated. In the former case, a definite rate of regeneration up to 48 h was observed, followed by gradual disintegration. In the latter case, there was no differentiation at all and by 96 h all had disintegrated. Hydras treated in 5 µg/ml AD did not disintegrate till 10 days.

When 24 h pretreated animals in 10 µg/ml AD brought back to normal solution after amputation, differentiation started after 48 h. The 48 h pretreated animals differentiated after 72 h (Figure 2, a). The 72 h pretreated animals did not differentiate but continued to live for 10 days or more (Figure 2, b) and no regeneration occurred in 96 h pretreated animals and all disintegrated beyond 4 days.

Decapitated hydras treated with AD at 10 µg/ml up to 48 h could not totally suppress the initial reconstitution process, but animals pretreated more than 48 h before amputation failed to regenerate. This indicates that although there is a burst of RNA synthesis at the early hours of hypostome determination[6], it is not really essential for the initial reconstitution process. The initial differentiation of the proximal end of hydra cut at the subhypostomal level is perhaps accomplished by structural proteins, synthesized with the help of a stable variety of messanger RNA. The existence of a stable mRNA or a masked templet material for initial hypostome determination in hydra has been respectively suggested by CLARKSON[7] and DATTA[5]. A masked RNA has also been reported to be responsible for the AD resistant protein synthesis in *Arbacia* egg[9]. From the above results it was revealed that the differentiation could only be suppressed if treatment with AD was done more than 48 h before amputation. This could perhaps be the time required for total turnover of the preexisting stable mRNA associated with the initial determination process. Treatment for more than 48 h in 10 µg/ml AD possibly inflicts permanent damage on the metabolic activities of the cell, as a result of which no regeneration took place[10].

Zusammenfassung. Actinomycin D-Wirkung auf die Mund-Tentakel-Regeneration bei *Hydra vulgaris*. Auch später absterbende Tiere beginnen diè Regeneration, was als Folge einer stabilen RNS angesehen wird.

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The Effects of Passive muscle Stretching on the Discharge of Individual Phrenic Motoneurones

Two mechanisms are discussed for the rapid change of ventilation at the start of muscular excercise: 1. direct stimulation of the respiratory centers by impulses from the brain during active excercise, 2. indirect reflex-induced stimulation of the respiratory centers attributed to the action of receptors of the limbs[1]. The purpose of this work was to evaluate the effect of stimulating muscle spindles and tendon organs on respiratory activity, while avoiding secundary effects induced by respiratory changes. We therefore investigated the response of phrenic motoneurones on passive muscle stretching in anaesthetized and paralyzed dogs under constant artificial respiration.

Methods. 5 dogs were anaesthetized with pentobarbitone sodium (Nembutal Abbott) 30 mg/kg i.v. After insertion of an endotracheal tube, spontaneous ventilation was abolished by continuous infusion of Gallamine triethiodide (Flaxedil Abbott). Artificial ventilation with pure oxygen was maintained by a respirator. Both vagi had been cut. The roots of the phrenic nerves on the two sides were dissected free in the neck and cut. Thin filaments were separated from the cut central end of the phrenic nerve on one side, subdivision usually beeing carried out until only a single responding motor unit was present. For stretching the muscles gastrocnemius and flexor digitorum superficialis, their tendons were cut from the calcaneal tuber and connected to the piston-rod of an airmotor (Bellows Valvair Corp.) with steel wire. The stretch-lenght was 35 mm in all experiments.

Results. Figure 1 shows some characteristic features of the response of phrenic motoneurones before, during and after stretching the calf muscles. During the whole procedure the arterial PCO_2 was kept at 37.8 Torr constantly. Before the onset of stimulus (upper row of the figure) one can see recurrent trains of 2 units separated by silent periods, with the smaller unit firing at the end of the inspiratory phase. At the onset of stimulation-indicated by the incline of the lower trace of the record the recruitment of an additional unit in the first poststimulus discharge is evident along with an increase of the discharge of the smaller unit.

With prolongation of the stimulus (second row), the frequency of cycles increases and the duration of discharges are shortened. Soon afterwards the frequency of the cycles decreases and the duration of discharges is again longer. By the end of stimulus (third row), the number of impulses in both the additional unit and the smaller unit is diminished. With the offset of the stimulus-indicated by the decline of the lower trace, there is again brief increase in the activity of phrenic motoneurones. Immediately afterwards the activity subsides and only one unit remains firing. In Figure 2 the frequency of all impulses per cycle and the duration of each discharge phase of the above experiment are plotted against time. In the relaxation phase at the beginning the average value of impulse frequency is about $15 \times sec^{-1}$, meanwhile the discharge phase lasted about 2 sec. The respiratory rate at this time was $12 \times min^{-1}$. After stretching the muscles one can differentiate 2 phases of a response of the phrenic motoneurones to the peripheral stimulus. In the first phase with the onset of stimulus – pointed

[1] P. DEJOUR, in *Handbook of Physiology*, 2nd edn (Ed. W. O. FENN and H. RAHN; Am. Physiol. Soc., Washington 1964), Section III, vol. 1 ,p. 631.

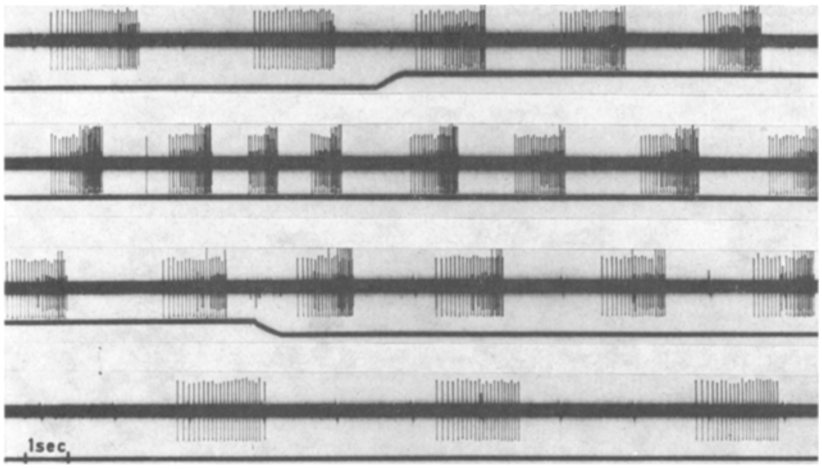

Fig. 1. Response of individual phrenic motoneurones on stretching the calf muscles to an extension of 35 mm. The onset of stimulus is indicated by the incline, the offset by the decline of the lower trace. Original recordings from the experiment shown in Figure 2.

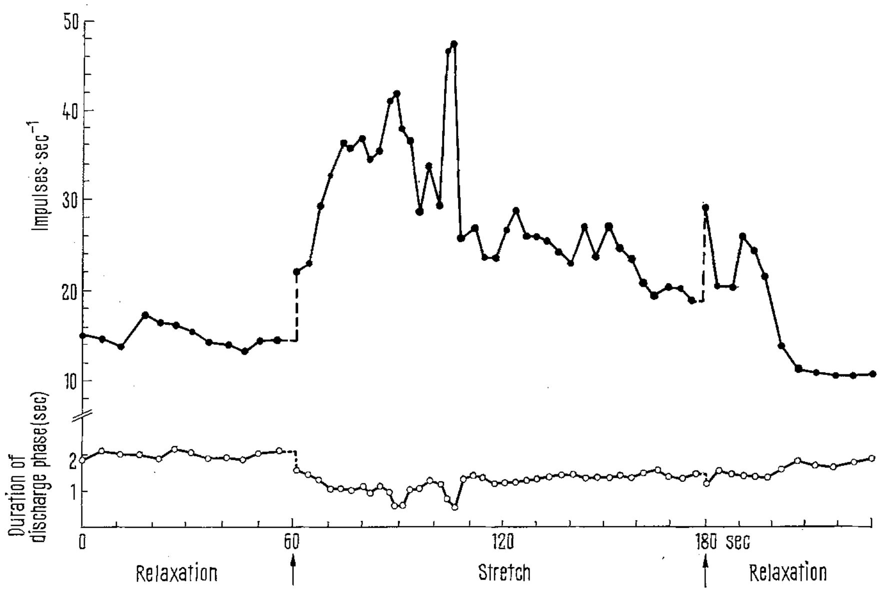

Fig. 2. Average discharge frequency (impulses $\times$ sec^{-1}) and duration of discharge phase (sec) in single phrenic motoneurones before, during and after muscle stretch. Arrows indicate onset and offset of stimulus.

out by the first arrow – the impulse frequency abruptly increases from 14.5 $\times$ sec^{-1} to a maximum value of 47.7 $\times$ sec^{-1} within a period of 46 sec. This increase is accompanied by a decrease of the duration of discharge phase from 2.1 sec to 0.6 sec at the maximum of impulse frequency. In this initial phase of stimulation of the muscle and tendon receptors, the rate of cycles also increases from 12 $\times$ min^{-1} to 21 $\times$ min^{-1}. As the stimulus continues the impulse frequency decreases in a second phase and reaches an almost steady state with values higher than during relaxation. In this second phase the duration of discharge increases to values about 1.4 sec. These values are clearly different from those before stretch. The frequency of the cycles in the second phase was 18 $\times$ min^{-1}-scarcely different of the first phase. With the offset of stimulus (second arrow) the impulse frequency temporarily increases again in the first 10 sec and declines finally to values about 11.1 $\times$ sec^{-1}. Duration of discharge phase increases and reaches values almost the same as in the beginning. The frequency of cycles in this period of relaxation was about 13 $\times$ min^{-1}.

Conclusion. From the results of the present experiments the following conclusion may be drawn: since phrenic motoneurones are activated by muscle stretching, it is possible that the involved muscle receptor afferents may influence the ventilatory control in muscular excercise[2].

Zusammenfassung. An narkotisierten, gelähmten und mit Sauerstoff beatmeten Hunden konnte gezeigt werden, dass passive Muskeldehnung reflektorisch die Impulsfrequenz einzelner Motoneurone des *N. phrenicus* erhöht und zur Rekrutierung neuer Einheiten führt.

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