been reported that after the injection of dextran, a compound is formed in the blood which differs from dextran and which, once transferred to normal animals, induces the AE[10]; however, there is some disagreement on this point[11].

It appears that hypophysectomy inhibits the AE and that the reactivity to Fe-Dex is restored by the transfusion of plasma of normal but not of hypophysectomized or resistant animals[12].

Résumé. Chez le rat albino de souche Sprague-Dawley, l'ablation de l'hypophyse empêche le développement de l'œdème anaphylactoïde provoqué par le dextran ferrique. Après hypophysectomie, la transfusion de plasma d'un rat normal de la même souche rend l'animal de nouveau sensible à l'agent anaphylactoïde. Cet effet n'est pas obtenu par la transfusion de plasma d'un rat Sprague-Dawley hypophysectomisé ou d'un rat de souche Wistar-Furth, qui est insensible à l'action anaphylactoïde du dextran ferrique.

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Presynaptic Inhibition in Crustacean Muscle: Axo-axonal Synapse

Most crustacean muscles are innervated by one or more inhibitory axons which regulate muscle tension. When active, the inhibitors weaken or abolish the contractions set up by the motor axons[1]. The inhibition may be either postsynaptic (i.e. the inhibitory transmitter substance acts directly on the muscle fibre membrane), or presynaptic (i.e. the inhibitory action is on the presynaptic terminal of the motor axon). Both effects occur simultaneously in some muscles[2]. In crustacean material the presynaptic effect has been demonstrated in only a few leg muscles of crayfish and crabs, although presynaptic inhibition is known in other material, particularly the mammalian central nervous system[3]. The phenomenon was first described in crustaceans by DUDEL and KUFFLER[2], who found that an inhibitory impulse timed to arrive 1–6 msec before a motor impulse reduced the quantal release of the excitatory transmitter substance while leaving the quantum size unchanged. Subsequent work[4–8] has supported the hypothesis[2] that the inhibitory effect arises from a reaction of the inhibitory transmitter with receptors on the motor axon terminal. The reaction induces an increase in chloride ion permeability of the axon membrane and a consequent decrease in amplitude of the motor impulse in the axon terminal. This reduced 'nerve terminal potential' (n.t.p.) is presumably less effective in releasing the excitatory transmitter.

So far the morphological basis for the effect has remained undisclosed. It could arise from inhibitory synaptic contacts on the motor axon terminal, or from leakage of transmitter from inhibitory neuromuscular synapses to adjacent motor axon terminals. All that can be said from previous histological evidence is that the inhibitory and excitatory nerve terminals occur close together[9]. In this report we present electron microscopical evidence for the existence of inhibitory synaptic contacts on the motor axon terminal.

The electrical manifestations of crustacean presynaptic inhibition are illustrated in Figure 1. The top traces in both records were obtained with an extracellular microelectrode placed close to an excitatory neuromuscular synapse to record the motor n.t.p. and the subsequent flow of current into the postsynaptic membrane (measured as a potential drop in the extracellular solution). The bottom traces show the postsynaptic potentials recorded across the muscle fibre membrane with an intracellular microelectrode. The recording methods are similar to those of DUDEL and KUFFLER[2]. Stimulation of an inhibitory axon (B) produces an inhibitory n.t.p., together with reduction of the excitatory postsynaptic potential, the motor n.t.p., and the synaptic current (compare A and B). The last 2 effects are seen only when the inhibitory n.t.p. precedes the motor n.t.p. by 1–6 msec.

Muscle fibres and attached nerves from preparations manifesting presynaptic inhibition, as shown in Figure 1, were fixed for electron microscopy 1 h in 4% glutaraldehyde in Millonig phosphate buffer, then washed in the buffer alone for 2 h and post-fixed 1 h in 1% buffered osmium tetroxide. The material was dehydrated in an acetone series and embedded in Durcupan (Fluka AG). Sections 50–150 nm in thickness were cut serially from regions thought to contain motor synapses, stained with ethanolic uranyl acetate, and examined in a Phillips EM 200 electron microscope.

Two types of nerve terminal were seen: 1 with predominantly round vesicles of fairly uniform size and 400–500 Å in diameter; and another with vesicles of slightly smaller size (often 200–400 Å) and less regular shape – sometimes round, but quite often elliptical, pear-shaped, or indented (Figure 2).

In the first type of ending, about 90% of the vesicles were circular in cross section, but in the second type, only 40–50% had this property. A statistical comparison of the 2 populations of vesicles was made by measuring the

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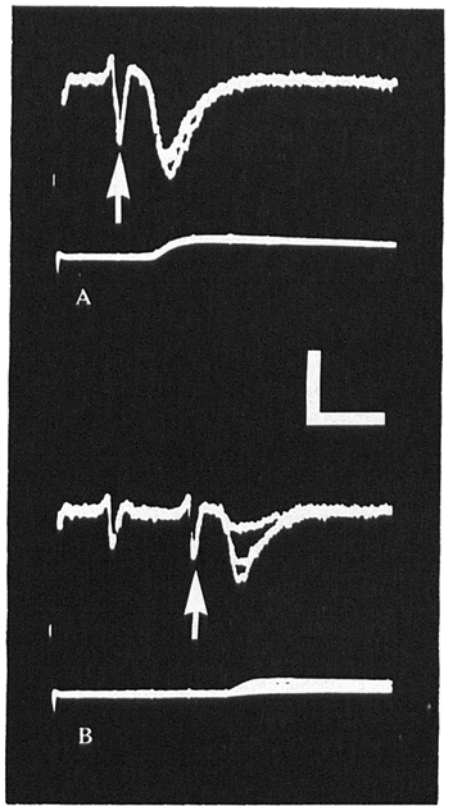

Fig. 1. Presynaptic inhibition in the stretcher muscle of the crab, *Pachygrapsus*. External recordings of synaptic currents and nerve terminal potentials (n.t.p.'s) appear in the upper traces, and internally recorded postsynaptic potentials in the lower traces. (A) Responses to 3 successive stimuli to the excitatory axon at 1/sec; the motor n.t.p. is indicated by an arrow, and is followed by the synaptic current record. (B) Arrival of inhibitory impulses (note inhibitory n.t.p.) just before the motor impulses, effects reduction of the motor n.t.p. (arrow), the synaptic current and the postsynaptic potentials. Calibration: voltage, 400 μV (top), 20 mV (bottom); time, 4 msec.

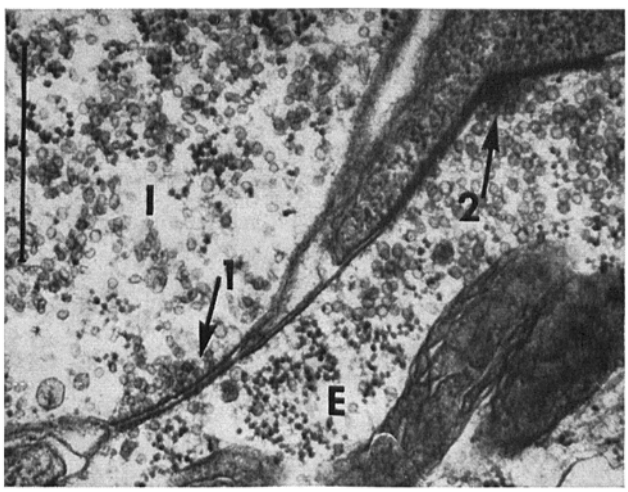

Fig. 2. Electron micrograph of presumed excitatory (E) and inhibitory (I) nerve terminals in the leg opener muscle of the crayfish *Orconectes*, showing a synaptic contact between the 2 axons (1), and an excitatory neuromuscular synapse (2). Note the large number of spherical vesicles in the E axon, and the less uniform vesicles in the I axon. Scale mark, 1 μ.

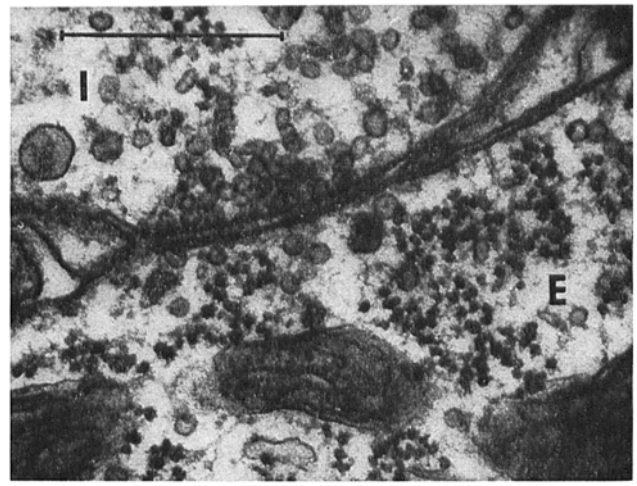

Fig. 3. Enlarged view of the axo-axonal synapse of Figure 2, showing the electron-dense synaptic membranes, the synaptic gap, the accumulation of vesicles in the I axon, and the lack of a corresponding accumulation in the E axon. Scale mark, 0.5 μ.

minimum width of 100 vesicles from each nerve ending. For the first type of ending, the mean width was 445 Å, with a standard deviation of $\pm$ 47 Å. The corresponding values for the second type of ending were 330 Å and $\pm$ 75 Å. The difference between the mean values was statistically significant at the 1% level. The smaller mean and larger standard deviation for the second type of ending reflects the larger proportion of non-circular vesicles.

Endings similar to these in some respects have recently been reported by UCHIZONO[10] to occur in crayfish leg muscles. In its possession of many non-spherical synaptic vesicles, the second type of ending bears a resemblance to the inhibitory endings on the crayfish stretch receptor neuron[10]. The first type of ending has been found[11] in crayfish and crab muscle fibres with no or very sparse inhibitory innervation; thus there is good reason to believe that this type of ending is excitatory.

In preparations of muscles which showed presynaptic inhibition, both types of ending were observed to form synaptic contacts with the muscle fibre. The synapses were characterized by electron-dense areas on both pre- and postsynaptic membranes, with an intervening gap of about 200 Å. The synaptic vesicles were often clustered densely right at the presynaptic membrane (Figure 2). The synapses often occurred along the axons at close intervals (1–3 μ). Typically, they were made with arms of the muscle fibre containing granular sarcoplasm and mitochondria, but devoid of contractile filaments.

In addition to the neuromuscular synapses, axo-axonal contact was observed (Figures 2, 3). Darkening of both axonal membranes occurred at the area of contact, and a gap of about 200 Å was present between the darkened membranes. In the axon thought to be inhibitory, synaptic vesicles were clustered near the membrane, but no such aggregations were seen in the excitatory axon. The region of contact clearly shows the structural features currently thought to be characteristic of chemically transmitting synapses. Furthermore, the aggregation of vesicles exclusively in the inhibitory axon indicates that this

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synapse is designed to transmit from the inhibitory to the excitatory axon.

The existence of inhibitory synapses on the motor nerve terminals clarifies the physiological observations. The minimum time of about 1 msec for the appearance of presynaptic inhibition following the arrival of the inhibitory impulse is consistent with a process involving a single chemically transmitting synapse, but too slow for typical electrical transmission[3] and probably too fast for diffusion of transmitter from the inhibitory neuromuscular synapses. We suggest that axo-axonal synapses are the agents of presynaptic inhibition in crustacean muscle[12].

Zusammenfassung. Es ist anzunehmen, dass die präsynaptische Hemmung der Crustaceen-Muskeln durch Reaktion eines hemmenden Überträgerstoffs mit Rezeptoren der Endigungen der motorischen Nervenfasern zustande kommt. Synaptische Kontakte des hemmenden Axons mit den Endigungen der motorischen Nervenfasern sind mit dem Elektronenmikroskop beobachtet worden. Wahrscheinlich vermitteln diese Strukturen den präsynaptischen Effekt.

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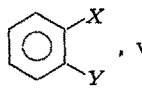
[12] We thank H. JOHNSTON for technical assistance. The study was supported by grants from the National Research Council of Canada and the Muscular Dystrophy Association of Canada.

Chemical Structure and Biological Activity of *o*-Disubstituted Derivatives of Benzene

We suggested in previous reports[1,2] the four-parameter equation (1) expressing a quantitative relation between the structure of *m*- and *p*-disubstituted benzene derivatives and the magnitude of their biological effect.

$$\log[LD_{50}]_{HH} - \log[LD_{50}]_{XY} = b_X + b_Y + e_X \times e_Y \qquad (1)$$

The symbol $\log[LD_{50}]_{XY}$ means logarithm LD_{50}, expressed in m*M*/kg body weight of the benzene derivative with substituents *X* and *Y*; $\log[LD_{50}]_{HH}$ is the same quantity for benzene.

We wish to report here our results obtained with a series of substances of type  , where *X* and *Y* represent H, CH_3, Cl, OH, NO_2, NH_2; the series of substances studied included all possible combinations of *X* and *Y*. The statistical treatment of the experimental data proved that *o*-disubstituted benzenes do not fulfil (1) because no $e_{o\text{-}X}$ exist, which satisfies the whole set of data. However, for *o*-disubstituted benzenes which satisfy the condition $X = Y$, interesting results were obtained. The data for these substances fit (2).

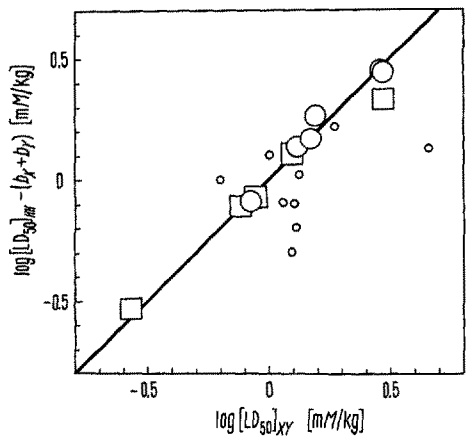
$$\log[LD_{50}]_{HH} - \log[LD_{50}]_{o\text{-}XX} = 2b_{o\text{-}X} \qquad (2)$$

where $b_{o\text{-}X} = b_{m\text{-}X} = b_{p\text{-}X}$ (see [2]). For substances where $X \neq Y$, the toxity is, in most cases, lower than that given by $b_X + b_Y$.

The data fitting (2) can be added to the series of *m*- and *p*-disubstituted benzene derivatives for calculating more precise b_X constants. These new data of b_X are summarized in the Table[3].

X	NO_2	Cl	OH	CH_3	H	NH_2
b_X	0.503	0.294	0.273	0.182	0.007	0.071

The correlation of measured toxicities with calculated values are shown in the Figure[4].



The toxicity data (LD_{50}) were determined for white mice (weight 20 ± 2 g) by the Thompson method. The substances were administered i.v. in a 20% polyvinylpyrrolidone[4] solution. □ *o*-derivatives of benzene where $X = Y$; o $X \neq Y$ respectively; ○ mono-substituted derivatives.

Zusammenfassung. Es wurden die i.v. LD_{50} einer Gruppe *o*-disubstituierter Benzolderivate, welche alle Kombinationen der erwähnten Substituenten enthielt, bestimmt. Die quantitative Korrelation zwischen chemischer Struktur und biologischer Aktivität wird in einer Gleichung gefasst.

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[4] Acknowledgment. We should like to thank the Badische Anilin und Soda-Fabrik for a sample of polyvinyl-pyrrolidone (Kollidon® biologisch geprüft).