Citellus lateralis. In the later species, lesions of the raphe nucleus discrupt hibernation and brain serotonin levels decrease during entrance into hibernation[14]. In *Cricetus cricetus,* 5HT levels of various parts of the brain are lower during winter in the hibernating animal than in the active one[10]. The high synthesis of 5HT we show in autumn may perhaps be related to its influence in provoking a preparation for hibernation which takes place during that season. NA is implicated in b. wt regulation[6]. At 15 °C, food intake of *Cricetus cricetus* is lower in autumn and winter than in spring and summer[8]. NA metabolism is also lower in autumn and winter than in spring and summer (table). The role of serotoninergic and noradrenergic pathways are not yet clearly understood, but in view of the role of this neurotransmitters in the b. wt regulation[6] and in normal sleep[15], the seasonal changes in NA and 5HT metabolism do appear important for the comprehension of the circannual rhythms of many physiological functions of the hibernators.

Levels of noradrenaline (NA_0) and serotonin ($5HT_0$) in various parts of the brain of the European hamster kept under constant conditions of light (light/dark 12/12) and temperature (15 °C)

	Autumn	Winter	Spring	Summer
Pons medulla				
NA_0 (ng/g)	640 ± 42	651 ± 71	583 ± 45	642 ± 41
Tt (h)	8.20	13.10	9.40	7.0
TR (ng/h)	78 ± 11.4	50 ± 17	62 ± 12.7	81 ± 18
$5HT_0$ (ng/g)	964 ± 127	984 ± 90	1072 ± 94	942 ± 93
Tt (h)	1.25	2.18	1.71	1.82
TR (ng/h)	620 ± 111	450 ± 41	628 ± 45	550 ± 75
Hypothalamus				
NA_0 (ng/g)	939 ± 103	899 ± 105	785 ± 85	849 ± 62
Tt (h)	7.0	14.90	10.10	5.65
TR (ng/h)	134 ± 28	60 ± 26	78 ± 27	150 ± 24
$5HT_0$ (ng/g)	1113 ± 87	1099 ± 93	1421 ± 167	1223 ± 155
Tt (h)	2.20	2.60	2.60	2.60
TR (ng/h)	508 ± 86	429 ± 49	550 ± 72	478 ± 70
Anterior telencephalon				
NA_0 (ng/g)	371 ± 18	373 ± 26	405 ± 38	433 ± 37
Tt (h)	7.50	17.30	8.85	7.25
TR (ng/h)	49 ± 4.9	21 ± 8.7	46 ± 11	60 ± 15.7
$5HT_0$ (ng/g)	586 ± 81	613 ± 45	636 ± 45	565 ± 53
Tt (h)	1.88	2.08	1.44	1.60
TR (ng/h)	313 ± 49	295 ± 57	441 ± 37	353 ± 46
Amygdala				
$5HT_0$ (ng/g)	853 ± 181	803 ± 112	918 ± 100	886 ± 120
Tt (h)	1.53	2.03	2.2	2.12
TR (ng/h)	558 ± 93	396 ± 53	417 ± 68	418 ± 57
Hippocampus				
$5HT_0$ (ng/g)	497 ± 37	568 ± 22	548 ± 46	502 ± 69
Tt (h)	1.76	3.05	1.44	1.78
TR (ng/h)	281 ± 37	186.6 ± 26	381 ± 52	283 ± 47

Tt, turnover; TR, turnover rate. Each value is the mean ± SE of 7 animals.

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Induction of electrical excitability in crustacean muscle by 4-cyclopentene-1,3-dione[1]

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Summary. 4-Cyclopentene-1,3-dione induces electrical activity in inexcitable crustacean muscle. This effect is blocked by previous treatment with p-chloromercuribenzoic acid. These results suggest that crustacean muscle becomes excitable when certain $-CH_2-SH$ side chains are converted to thioethers having carbonyl groups.

Studies of the effects of sulfhydryl reagents on the functional properties of excitable membranes led to the general conclusion that blocking free SH groups causes the loss of electrical excitability[2-9].

Recently we investigated the action of the SH reagent NEM on inexcitable crustacean muscle fibres and our results were opposed to those previously reported[10,11]. Electrical excitability was induced in this tissue by treatment with NEM (1-2 mM; 5-10 min). This effect is not simply the result of binding free SH groups, since organic mercurials did not induce excitability, though they prevented the subsequent effect of NEM. In an attempt to determine the structural features of NEM necessary to induce excitability, we found that the ethylene chain attached to the N was not needed, since other N-maleimide derivatives also exerted the same action[11]. This suggested that the 2 symmetrical carbonyl groups or the tertiary nitrogen may be more important. To study the role of these groups, we performed experiments with 4-CPD, a compound similar to maleimide but with a methylene group instead of the nitrogen. Although it has not been used as an SH reagent, it has a double bond capable of reacting with free SH groups[12].

Materials and methods. Experiments were performed on the ventroabdominal flexor muscles of *Atyas occidentalis.* The muscles were fixed on a Petri dish provided with a layer of Sylgard and filled with Van Harreveld's saline[13]. The

transmembrane potential was recorded with conventional glass microelectrodes filled with 3 M KCl. The potential across the muscle membrane was changed by injecting current through a second microelectrode at less than 100 µm from the recording one. The applied current was monitored in the second beam of the CRO.

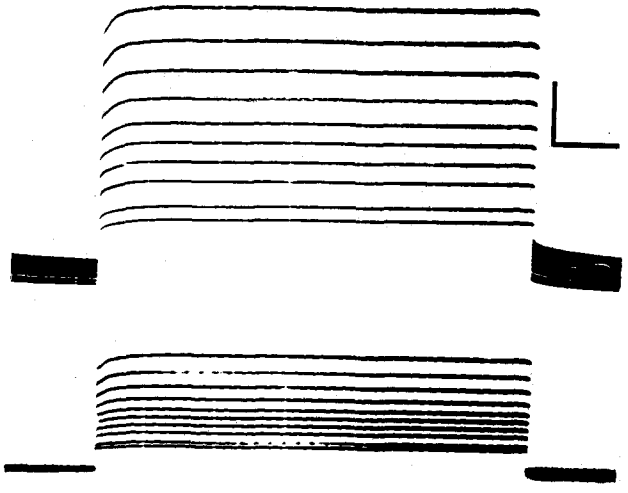

Fig. 1. Inexcitability of *Atyas* muscle in normal Ringer. In this and the following figure, the lower tracings are records of the depolarizing current and upper tracings are membrane potential. Calibration: vertical, 20 mV and 2×10^{-6} A; horizontal, 100 msec.

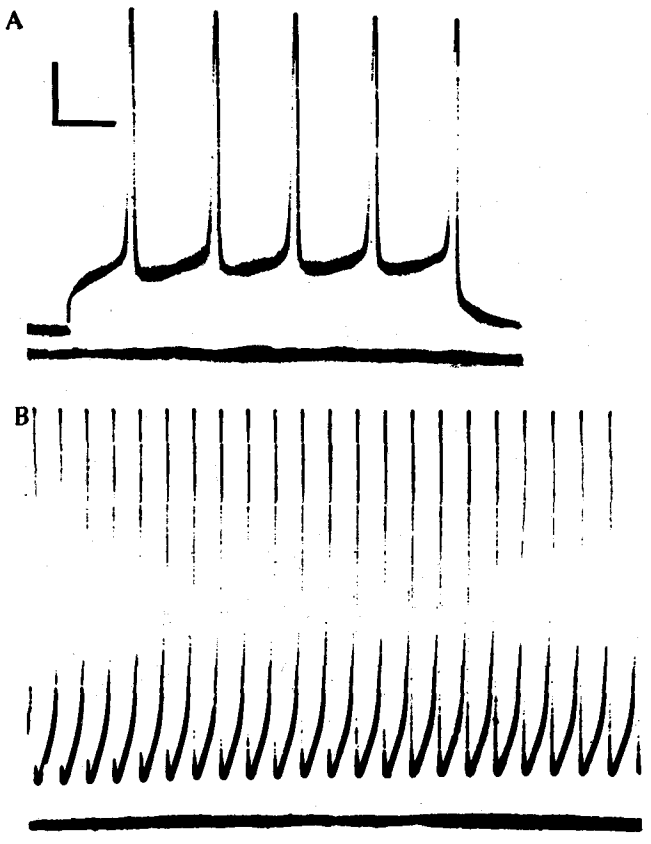

Fig. 2. Repetitive spike activity induced by 4-CPD. *A* Rhythmic generation of action potentials by a small depolarization. *B* Train of spikes produced by the intracellular injection of a depolarizing current of about 3×10^{-7} A. Calibration: vertical, 10 mV and 2×10^{-6} A; horizontal, 100 msec (A) and 500 msec (B).

Results and discussion. Figure 1 shows the passive electrical behaviour of the muscle membrane before treatment with 4-CPD. 10 catelectrotonic potentials were elicited by injecting outward current pulses into a muscle fibre of *Atyas* immersed in normal saline. Even though the fibre was depolarized by up to 70 mV (on a resting potential of 75 mV) there are no indications of active electrical responses.

In contrast to figure 1, figure 2 shows rhythmic firing of action potentials in fibres of the same muscle after exposure to 2 mM 4-CPD for 5 min. Record A was taken from a fibre with low electrical threshold which fired spikes when depolarized about 5 mV above the resting potential. Record B, shows spikes of about 60 mV elicited in another fibre by a depolarization of 20 mV.

To determine whether the effect of 4-CPD is due to its ability to combine with SH groups we also tested its saturated analog 1,3-cyclopentanedione. This compound did not induce excitability even when used at a concentration of 3 mM for periods of up to 15 min.

In other experiments, we blocked the free SH groups by exposing the muscle to the organic mercurial PCMB for 5 min before treatment with 4-CPD. This prevented the action of 4-CPD. The blocking effect of PCMB could be reversed by exposing the preparation to cysteine (5 mM; pH 7.85; 10 min). If, following this treatment, the muscle is immersed in a 4-CPD solution, electrical excitability appears.

These observations suggest that 4-CPD most likely exerts its excitability-inducing effect by combining with free SH groups, and that no tertiary nitrogen is needed for it to occur. They lend support to our hypothesis that the appearance of excitability in crustacean muscle depends on the conversion of $-CH_2$-SH side chains to thioethers having carbonyl groups[11]. Such a thioether represents a different side chain having new reactivity and functional properties. The new carbonyl groups may interact with neighboring amino groups through hydrogen bond formation or by acylation. The new side chains, formed by either NEM or 4-CPD, may lead to the formation of bonds between different regions of a protein or between different protein units. Excitability properties may be changed either by inducing conformational changes or by preventing the occurrence of them.

Abbreviations: NEM, N-ethylmaleimide; 4-CPD, 4-cyclopentene-1,3-dione; PCMB, p-chloromercuribenzoic acid.

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