breaks, and exchanges were found at metaphase and bridges at anaphase. The presence of the latter types of aberrations indicates a prior reunion of broken chromosome ends. Treatment for 2, 3, or 4 h with immediate fixation at the end of the treatment time yielded breakage and some reunion. These results provide significant support for a post-synthetic effect of 5-FUDR.

Cells treated for 1 h or more and fixed 12 h (but not as late as 24 h) exhibited numerous breaks, but less reunion of broken ends than was found in cells fixed earlier than 12 h. Thus, breaks can be induced both during and following the period of DNA synthesis, and less reunion occurs during the period of synthesis than during post-synthetic periods. In addition, the earlier the time of treatment during the cell cycle, the greater is the level of mitotic inhibition. Roots treated for 1, 2, or 3 h and fixed immediately thereafter had almost as many mitotic figures as found in the control slides (Figure 1). Most other treatments resulted in severe mitotic inhibition. Thus, the inhibition of mitosis also appears to be greatest when the cells are treated during or close to the period of DNA synthesis. In conclusion, evidence has been obtained to support the contention that 5-FUDR produces cytological damage not only during the synthetic period in the cell cycle, but also during the post-synthetic period and during early stages of mitosis. At the same time, reunion of broken ends of chromosomes is greatly reduced, especially when cells are exposed during DNA synthesis or close to that period.

Conclusions. Several interesting comparisons between the effects of 5-FUDR and hydroxylamine are possible. From the standpoint of radiomimesis, both 5-FUDR and hydroxylamine produce the kinds of cytological damage to be expected. Of particular interest is the fact that their specific effects in the cell are based upon quite different chemical activities. Hydroxylamine appears to affect the base composition of DNA, while 5-FUDR results in the inhibition of DNA synthesis. Yet both agents are capable of causing breaks in the chromosomes near or during the period of DNA synthesis as well as during post-synthetic periods. However, hydroxylamine produces drastic effects in a few cells, whereas 5-FUDR affects a larger cell population. The apparent inhibition of rejoining (restitution and reunion) by either agent is difficult to explain. There is evidence that nucleotide synthesis is required for reunion of breaks induced by 5-FUDR[9], as might be expected when the breaks occur during the synthetic period. However, the rejoining of breaks induced at a later time may not be related to DNA synthesis. Rejoining of breaks induced during late interphase or prophase may be somewhat impaired because of chromosome movements, but it seems unlikely that this alone can account for the considerable absence of reunion or restitution. Since many of the treatments involved rather long exposures to the agent used – 1 h or longer – one might expect that the breaks were induced throughout this treatment period such that relatively few were available for reunion at any one time. However, studies with other radiomimetic agents do not support such a contention. In addition, the implication that these two particular agents produce more extensive damage in the chromosomes because of their specific chemical effects on the structural components of the chromosomes (presumably mainly DNA), may explain the lack of rejoining.

Among the more significant conclusions to be drawn from these studies are: (1) 5-FUDR treatments suggest the importance of DNA metabolism in the phenomena of restitution and reunion, (2) hydroxylamine is a radiomimetic agent that apparently has as its basis of effect specific influence on the cytosine-guanine pairs of the DNA molecule. This latter conclusion has important implications in terms of the organization of the chromosome, since the majority of damage appears to have occurred in the heterochromatic regions of the chromosomes. Finally, it should be indicated that DNA is not the only structural component of the chromosomes, but that histone protein is also quite important. Thus, chromosome breakage must certainly involve disruption of protein along with DNA. Current investigations are concerned with this aspect of radiomimesis[13].

Résumé. L'hydroxylamine et la 5-fluorodeoxyuridine provoquent des altérations notables dans les chromosomes de méristèmes radiculaires (*Vicia* et *Allium*). Ces deux agents empêchent aussi, dans une large mesure la réunion des segments après ruptures des chromosomes. Il est probable que l'hydroxylamine affecte spécifiquement les régions hétérochromatiques.

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γ-Aminobutyric Acid and Crab Muscle Fibres

The action of γ-aminobutyric acid (GABA) on crustacean muscle fibres has been investigated by several groups of authors[1–6]. In the case of crayfish[1] and lobster[2] muscle fibres, the application of GABA produces a marked increase in membrane conductance as does the transmitter substance of the inhibitor neurons. For crab muscles several authors[3–5] have reported no effect of GABA on membrane conductance although the substance blocks the excitatory junction potentials. However, in a recent note[6] it was demonstrated that GABA causes an increase in membrane conductance in fibres of the opening and closing muscles of *Cancer borealis*. This result is in conflict with that obtained by other workers in the closing muscle of the same species[5].

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The possibility that different types of muscle fibres in the same crustacean muscle may respond differently to GABA has not been previously considered. Recently the discovery was made that crab muscle fibres showing extreme differences in membrane properties can exist in the same muscle[7]. Experiments were therefore performed to test the effects of GABA on the different types of crab muscle fibre.

A small muscle called the accessory flexor muscle is found in the meropodite of the walking legs of crabs[8]. This muscle is closely associated with the large locomotor muscles, the main flexor and extensor, but is distinct from them and can be completely isolated by careful dissection. The accessory flexor muscle of the crab *Cancer magister* has been found to contain 'fast' or 'phasic', 'slow' or 'tonic', and 'intermediate' muscle fibres[9]. The 'tonic' muscle fibres are characterized by large membrane time constants, slow and sustained tension development, relatively small diameters, and by a distinct histological appearance in cross section which superficially resembles the 'Felderstruktur' of certain vertebrate muscle fibres. The 'phasic' muscle fibres, on the other hand, have small membrane time constants, rapid tension development, relatively large diameters, and a histological appearance in cross section resembling the 'Fibrillenstruktur' of many vertebrate muscle fibres[10]. The phasic and tonic muscle fibres are grouped in different parts of the accessory flexor muscle and can be readily identified visually and electrically.

GABA (10^{-6} to 10^{-5} g per ml) was applied to the muscle while square pulses of hyperpolarizing current were passed into individual muscle fibres by means of a stimulating microelectrode. The membrane voltage changes produced by the applied current were monitored by a recording microelectrode inserted into the muscle fibre within 100 μ of the stimulating microelectrode.

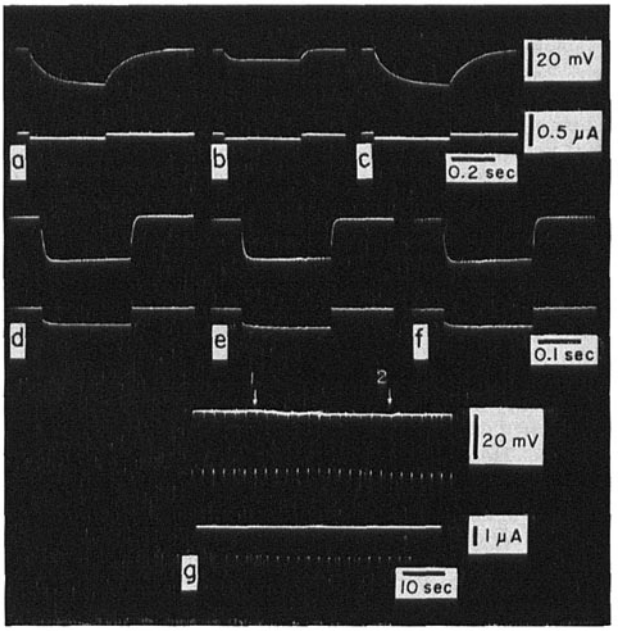
The effect of GABA on the tonic muscle fibres was always very pronounced. These fibres are characterized by relatively high membrane resistance (2000 to 20000 ohm cm^2) and by low resting potentials (50 to 60 mV). Within a few seconds of application of GABA the membrane resistance fell to about a quarter or even less of its initial value (Figure, a, b, c). The membrane time constant was greatly reduced, reflecting the decrease in membrane resistance. In addition, the resting potential was generally increased by about 5 mV. Clearly GABA produced a very marked increase in membrane conductance in these muscle fibres.

The phasic muscle fibres, which are characterized by relatively low membrane resistance (200 to 600 ohm cm^2) and by resting potentials of 65 to 80 mV, reacted differently to application of GABA. Typically a hyperpolarization of 1 to 3 mV was produced. However, even after several minutes of exposure to the chemical, very little change in membrane resistance or in membrane time constant was observed (Figure, d, e, f, g). The decrease in membrane resistance observed in these muscle fibres during treatment with GABA was usually about 10 to 15%. In many of the 'intermediate' type fibres the change was greater, but did not approach that observed in the tonic fibres.

In both phasic and tonic muscle fibres, the effects of GABA were apparent a few seconds after application of the chemical (Figure, g) and were rapidly reversed by normal saline.

The results indicate that different fibres in the same crab muscle can be affected to different extents by GABA. This finding may partly explain some of the conflicting results reported previously in the literature.

There is evidence that GABA may be the transmitter substance of the crustacean inhibitory axons[11], although this claim is disputed[12]. It was therefore of further interest to determine whether stimulation of the peripheral inhibitory axons could affect phasic and tonic crab muscle fibres differentially. The inhibitory axon of the accessory flexor muscle proved extremely difficult to prepare in isolation. Therefore, experiments were performed on other crab muscles, particularly on the closing muscle of *Chionecetes tanneri*, a deep-sea spider crab, and on the opening muscle of *Pachygrapsus crassipes*, a shore crab. In these muscles phasic and tonic muscle fibres, similar in many respects to their counterparts in the accessory flexor muscle of *Cancer*, could readily be found. The inhibitory axons could be stimulated separately from the excitatory axons. In both muscles the tonic fibres were observed to be affected by GABA to a greater extent than the phasic muscle fibres.



Examples of the effects of GABA on membrane voltage responses (upper traces) to square pulses of hyperpolarizing current (monitored in the lower traces). In (a) to (c) records were made from a tonic fibre before application of GABA (a), 10 sec after application of 5×10^{-6} g/ml GABA (b), and 2 min after restoration of normal saline (c). GABA causes a marked decrease in membrane resistance. (d–f) Records from a phasic fibre during a similar sequence of treatments; the effect of GABA on the membrane is small. (g) Effect of GABA (10^{-5} g/ml) on another phasic fibre: arrows indicate (1) application of GABA, and (2) return to normal saline.

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In the closing muscle of *Chionecetes*, stimulation of the inhibitory axon at low frequencies (5 to 20 per sec) caused appreciable hyperpolarization (5 to 10 mV) of tonic muscle fibre membranes, and a reduction of membrane resistance of about 30%, as determined by analysis of membrane responses to square pulses of hyperpolarizing current. Even single stimuli applied to the inhibitory axon resulted in large hyperpolarizing junction potentials in these fibres. By contrast, stimulation of the inhibitory axon at considerably higher frequencies (20 to 40 per sec) resulted usually in small hyperpolarizations (about 2 mV) in phasic muscle fibres, and in reduction of membrane resistance of only 5 to 15%. In addition, stimulation of the inhibitory axon during excitation of the 'slow' motor axon resulted in marked reduction in size of the excitatory junction potentials in tonic muscle fibres (α-inhibition)[13], whereas in phasic muscle fibres inhibitory stimulation had a negligible effect on the sizes of the 'slow' excitatory junction potentials.

Experiments on the opening muscle of *Pachygrapsus* yielded a similar pattern of results. Tonic muscle fibres showed pronounced hyperpolarization during low frequency stimulation of the specific inhibitory axon of the opening muscle, whereas no electrical activity of this nature was detectable in the phasic muscle fibres. During stimulation of the inhibitory axon at a given frequency, changes in membrane resistance were always much greater in tonic than in phasic muscle fibres. Stimulation of the inhibitory axon during a burst of impulses in the excitor axon produced strong α-inhibition in tonic muscle fibres. α-Inhibition was present, but much weaker, in phasic muscle fibres, and could be seen only when the frequency of the inhibitory stimulation was rather high (60 to 100 per sec).

The fact that stimulation of the inhibitory axons resulted in more pronounced α-inhibition and membrane resistance changes in tonic rather than in phasic muscle fibres could mean that the former are more densely innervated by the inhibitory axons. If this were the case, and if the effect of GABA is mainly activation of the subsynaptic muscle fibre membrane sensitive to the inhibitory transmitter substance, then an explanation for the more pronounced effect of GABA on tonic muscle fibres is available.

However, other factors may also be important. Consistently, the tonic muscle fibres have relatively low resting potentials, typically between 55 and 60 mV in the accessory flexor muscle of *Cancer* (Table). By contrast, the phasic muscle fibres have resting potentials close to 70 mV. Phasic and tonic muscle fibres in other crab muscles show a similar discrepancy in the resting potential measurements. It was thought that this difference might be due to different potassium and/or chloride contents in the two muscle fibre types. In the accessory flexor muscle it proved feasible to dissect out small bundles of phasic and tonic muscle fibres and to determine their potassium and chloride contents after washing away the extracellular fluid in isotonic sucrose[14]. Results of these analyses are summarized in the Table. It is apparent that the phasic and tonic muscle fibres have similar potassium contents, but that the tonic muscle fibres contain significantly more chloride.

Assuming values for external potassium and chloride of 10 mM and 530 mM, respectively, estimation of the potassium and chloride equilibrium potentials can be made using the Nernst equation: $E_k = 58 \log K_i/K_o$, and $E_{cl} = 58 \log Cl_o/Cl_i$. These estimates (Table) show that in phasic muscle fibres, the potassium and chloride equilibrium potentials are the same as the average membrane resting potential. In tonic muscle fibres, however, the potassium and chloride equilibrium potentials are both higher than the average membrane resting potential. This finding suggests that the tonic muscle fibre membrane, unlike the phasic, may be relatively impermeable to potassium and chloride in its resting state.

The action of the crustacean inhibitory transmitter substance (and of GABA) on the muscle fibre membrane is thought to involve an increase in membrane chloride conductance[1]. Since the tonic muscle fibres have resting potentials lower than the chloride equilibrium potential, the action of the inhibitory transmitter substance would be expected to result in marked hyperpolarization in these fibres. In phasic muscle fibres, little electrical activity would be expected, since the resting potentials are almost the same as the chloride equilibrium potential. In addition, the tonic muscle fibres, with lower initial membrane chloride conductances, can logically be expected to show a relatively large increase in membrane conductance during stimulation of the inhibitory axons or during application of GABA[15].

Zusammenfassung. Eine Reihe von Krabbenmuskeln zeigt zwei Arten von Muskelfasern: «phasische» und «tonische» mit sehr unterschiedlichen Membraneigenschaften. Die phasischen Muskelfasern zeigen verhältnismässig kleine Permeabilitätsänderungen der Membranen nach Zugabe von GABA oder nach Stimulierung des peripheren hemmenden Axons. Die tonischen Muskelfasern zeigen sehr deutliche Permeabilitätsänderungen nach Zugabe von GABA oder Stimulierung des hemmenden Axons. Die Grundlagen dieses unterschiedlichen Verhaltens werden analysiert.

Potassium and chloride ions in phasic and tonic muscle fibres of the accessory flexor muscle of *Cancer*. (Numbers in brackets indicate the range of values)

Muscle fibre type	Resting potential (mV)	Intracellular chloride Cl_i (mM)	Chloride equilibrium potential E_{cl} (mV)	Intracellular potassium K_i (mM)	Potassium equilibrium potential E_k (mV)
Phasic	71 (65–82)	32 (28–36)	71	167 (154–178)	71
Tonic	57 (51–65)	41 (34–50)	64	163 (147–172)	70

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