Results and discussion. The mean resting potential of hearts of 29 cardiac mutants was not statistically different from that found for 29 normal hearts (Table). The resting potential magnitude was similar to that found for other inexcitable tissues[5]. The 3 drugs in all concentrations tested did not alter resting potential magnitudes or induce myogenic activity in any mutant embryos, whereas the heart rates of stage 34 + normal embryos were modified by drugs[4]. Electrical stimulation strength, sufficient at times to stimulate somatic muscles, in the presence or absence of these 3 drugs did not alter resting potential magnitudes or induce myogenic activity in mutant embryo hearts. These results suggest that myogenic factors essential to produce action potentials are absent in homozygous gene *c* mutants, and their heart cell membranes may remain in a stage of development similar to that found prior to stage 34. Mutant embryonic hearts never showed myogenic activity during stages 34–38, in contrast to myogenic activity always present during stages 34–38 in normal embryonic hearts.

HUMPHREY[6] transplanted primordial hearts of mutants into homozygous (+/+) or heterozygous (+/*c*) normals and produced myogenic activity. Conversely, transplanting normal primordial hearts into mutant recipients inhibited myogenic activity. The factors or substances controlling initiation of myogenic activity do not appear to be present in body fluids and it seems to be restricted to the region of the heart since parabiosis of normals with mutant siblings failed to induce myogenic activity in mutants or block myogenic activity in normal animals. The experiments reported here also suggest that the 3 neurohumors used were not the natural substances essential to initiate myogenic activity.

Summary of the measured characteristics of in vivo hearts of normal and mutant *Ambystoma mexicanum*

Characteristic	Tissue	Genotype	Stages 34–38
Resting potential (mV)	Random cell sample	Normal	24 ± 2[a]
		Mutant	24 ± 2
Action potential (mV)	Ventricle	Normal	32.9 ± 2
	Ventricle	Mutant	[b]
Heart rate (beats/min)		Normal	75 ± 18
		Mutant	[b]

[a] Mean ± S.E.M. [b] None observed.

JACOBSON and DUNCAN[7] reported that developing hearts may be under the influence of 1. an inducer that increases heart differentiation, 2. an inhibitor that delays heart differentiation, and 3. a stimulator that increases beating heart formation. Embryos of mutant gene *c* may influence synthesis of only 1. and/or 2. which results in modulation of membrane properties essential for excitation, conduction and action potentials. The results reported here suggest that homozygous gene *c* mutants have cardiac cell membranes that are inexcitable. This may be due to differences in membrane permeability to ions essential for excitation. Low resting potential magnitudes may be due primarily to a higher sodium and potassium permeability ratio in nonexcitable cells[5]. The effects of homozygosity for gene *c* seem restricted to cardiac tissues since electrical stimulation evokes somatic movements without concomitant responses in cardiac cells. This lack of cardiac contractility may be attributed to insufficient myofibrillar material[3]. Large numbers of mitochondria and other intracellular structures associated with pathological states have been reported[3]. It is not yet clear whether these cellular pathological-like conditions reflect only intracellular changes and activities or also an alteration in membrane response properties.

Résumé. Le mutant homozygote d'*Ambystoma mexicanum* qui n'a pas d'activité cardiaque myogénique, est aussi réfractaire aux modifications chimiques et électriques des potentiels membranaires et de la fréquence cardiaque. Il diffère en cela de l'animal normal au même stage de developpement embryonnaire.

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[5] J. A. WILLIAMS, J. theor. Biol. *28*, 287 (1970).
[6] R. R. HUMPHREY, in preparation.
[7] A. G. JACOBSON and J. DUNCAN, J. expl. Zool. *167*, 79 (1968).
[8] Established investigatorship of the American Heart Association.
[9] Supported by U.S. Public Health Service Career Development award No. HE 11,929.

The Effect of pH on Quinine-Induced Contractures and Excitation-Contraction Coupling in Crustacean Skeletal Muscle

Although the action of quinine in both potentiating and depressing contractility of skeletal muscle has been known for some time[1,2], the ability of quinine to induce contractures has been only recently reported in frog[3] and crustacean[4] skeletal muscle. In one of these reports[3], it was noticed that changes in pH affected the contracture-induction ability of quinine, but the mode of action of pH changes during quinine activation, and the actual site of action of quinine on skeletal muscle are still not clear. This study of the effect of pH changes both on membrane potentials, quinine contractures and excitation-contraction coupling of crustacean skeletal muscle was carried out in an attempt to clarify the way in which quinine activates the contractile mechanism of skeletal muscle, and the modifying effect of pH on quinine activation.

Methods. The dactylopodite flexor muscle of the walking legs of the common shore crab, *Carcinus maenas* was used throughout this study. Details of procedure and preparations have already been published[4]. In some experiments, whole muscle preparations were used. Flexion of the dactylopodite was measured isometrically on a 50*g* strain gauge, the output of which was fed into a Tektronix 502A oscilloscope. Occasional experiments were carried out on isolated bundles of fibres dissected from the flexor muscle. These were mounted in a 5 ml perfusion chamber and tension was recorded isometrically on a 5 or 10*g* strain gauge. Membrane potentials of muscle fibres were measured with conventional 3*M* KCl electrodes, details of recording procedures have already been published[5]. The crab saline used was that of ATWOOD[6], containing (in m*M*), NaCl 520; KCl 10; $CaCl_2$ 11; $MgCl_2$ 8; $NaHCO_3$ 3, modified

by addition of 100 mM sucrose to ensure isotonicity with the muscle fibres. Quinine sulphate was made up as a stock solution (5 mM) in crab saline. The K^+ contracture saline was 200 mM K^+ crab ringer, the increase in KCl being compensated for by a corresponding reduction in NaCl. The pH of the quinine-crab saline was 7.3, and the pH was lowered by addition of HCl and raised by addition of $NaHCO_3$. Drug flow through the preparations was metered with micro peristaltic delta pumps.

Results and discussion. Quinine-induced contracture tension is considerably affected by the pH of the perfusion medium (Figure 1). Standard crab ringer with 5 mM quinine has a pH of 7.3, and if the pH is lowered, contracture tension is decreased, while if pH is raised, a significant augmentation of contracture tension is observed. Changes in pH had surprisingly little effect on the total duration of the quinine-induced contracture, all contractures remained phasic, but raising the pH markedly accelerated the rate of rise of contracture tension. In all of these experiments, the preparations were perfused for 10 sec with normal crab ringer at the pH value under investigation before the experimental quinine-crab ringer was perfused. It is clear that the effects of pH alteration are so rapid that any significant alteration of myoplasmic pH is very unlikely (see CALDWELL[7], BIANCHI and BOLTON[8], BIANCHI[9]). In view of this, it would seem that changes in the pH of the bathing medium must alter the interaction between quinine and the fibre sites on which quinine acts to induce tension. It has already been shown that the effect of quinine on reticulum[10–13] bears little relation to the gross effect of quinine on tension development in whole crab skeletal muscle[4], as is the case in some other muscles[1,2,11]. Quinine could activate crab skeletal muscle at the trigger (peripheral) stage of the excitation-contraction coupling cycle[4], unlike the action of caffeine on skeletal muscle[14,15]. The effect of changes in pH on quinine action may thus take place at a peripheral fibre site, during the early stages of the excitation-contraction coupling cycle. Since quinine may activate crab skeletal muscle by initiating membrane depolarization[4], the most likely initial parameter to be altered by pH-quinine interaction would be the membrane potential, so the effect of changes in pH on the muscle fibre membrane potentials was checked. The results of these experiments are summarized in the Table. It can be seen that changes in pH have little effect on the muscle fibres, even after 5 min perfusion with salines at radically different pH values.

Although quinine does not modify the mechanical threshold of crab skeletal muscle fibres[4], the possibility arose during these experiments that changes in pH may themselves modify the mechanical threshold, thus altering the quinine-induced contracture tension via a different route. The mechanical threshold of muscle fibre preparations was checked by eliciting potassium contractures (with 200 mM KCl crab saline) in single preparations

The effect of pH on membrane potentials of crab skeletal muscle fibres

pH	Resting potential (mV ± S.E. of mean)	Action potencial (mV ± S.E. of mean)
5.0	63.0 ± 4.1	68.0 ± 7.2
6.0	61.6 ± 5.3	65.6 ± 6.3
7.3	65.0 ± 2.8	71.4 ± 7.9
8.5	67.2 ± 3.4	74.0 ± 8.2

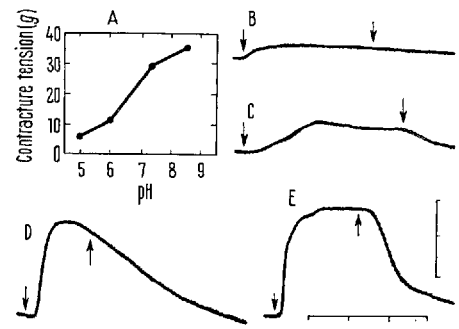

Fig. 1. The effect of the pH of the perfusion medium on the contracture tension induced by 5 mM quinine in crab saline. A) mean results from five separate experiments. B–E) results of typical experiments on a single whole muscle preparation. The preparation was perfused for 10 sec before the quinine was introduced. After the quinine washout, 30 min in normal crab ringer was allowed before the next treatment. B) pH 5; C) pH 6; D) pH 7.3; E) pH 8.5. Calibrations (20 g and 60 sec) apply to all traces.

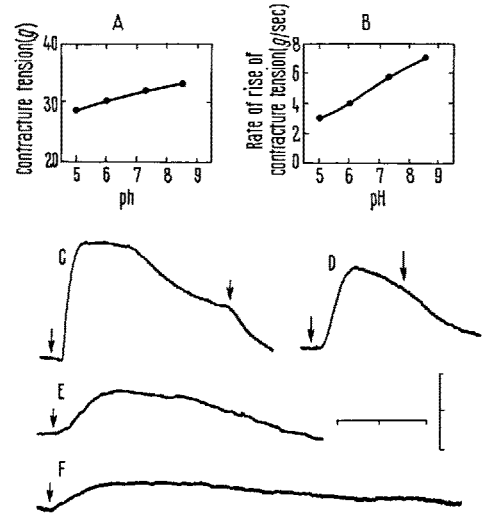

Fig. 2. The effect of pH on the K^+ contracture tension of whole muscle preparations. A) the effect of pH on total K^+ contracture tension. B) the effect of pH on the rate of rise of K^+ contracture tension. Each point in A) and B) is the mean of 5 records. C–F) results of experiment on a single preparation. C) pH 8.5; D) pH 7.3; E) pH 6.0; F) pH 5.0. All contracture salines contained 200 mM KCl. Calibration scales (20 g and 1 min) apply to all traces.

[1] A. M. HARVEY, J. Physiol. Lond. *95*, 45 (1939).
[2] W. LAMMERS and J. M. RITCHIE, J. Physiol., Lond. *129*, 412 (1955).
[3] A. ISAACSON, K. YAMAJI and A. SANDOW, J. Pharmac. exp. Ther. *171*, 26 (1970).
[4] H. HUDDART, J. Physiol. Lond., in press (1971).
[5] H. HUDDART, Comp. Biochem. Physiol. *18*, 131 (1966).
[6] H. L. ATWOOD, Comp. Biochem. Physiol. *10*, 17 (1963).
[7] P. C. CALDWELL, J. Physiol. Lond. *142*, 22 (1958).
[8] C. P. BIANCHI and T. C. BOLTON, J. Pharmac. exp. Ther. *157*, 388 (1967).
[9] C. P. BIANCHI, Fedn Proc. *27*, 126 (1968).
[10] A. ISAACSON and A. SANDOW, J. gen. Physiol. *50*, 2109 (1967).
[11] F. FUCHS, E. W. GERTZ and F. N. BRIGGS, J. gen. Physiol. *52*, 955 (1968).
[12] A. P. CARVALHO, J. gen. Physiol. *52*, 622 (1968).
[13] W. VAN DER KLOOT, Science *164*, 1294 (1969).
[14] A. SANDOW and M. BRUST, Biochem. Z. *345*, 232 (1966).
[15] H. HUDDART, Comp. Biochem. Physiol. *29*, 1031 (1969).

successively at pH 5, 6, 7.3, and 8.5. The potassium contracture method of measuring changes in mechanical threshold of muscle fibres has been extensively employed to illustrate this aspect of caffeine activation in vertebrate[16], crustacean[15] and insect[17] skeletal muscles. The results of typical experiments are shown in Figure 2. In these experiments, the muscle fibre preparations were perfused for 10 sec with normal crab ringer at the experimental pH, and the perfusate was then replaced by 200 m*M* KCl crab ringer at this pH. It is clear that reduction in pH noticeably lowers the rate of rise of the KCl contracture tension, but there was no significant effect of pH on the maximal contracture tension achieved. From these results we may rule out any effect of changes in pH on the actual mechanical threshold. Changes in pH would thus appear to have little effect on the quinine interaction with the excitation-contraction coupling process at the muscle fibre periphery.

Changes in pH do have an important effect on the physical state of some alkaloids in solution. Caffeine contractures increase in size with increasing pH of the medium[18], and it has been suggested that the variability of caffeine action may be due to the greater proportion of 'aggregated' caffeine in solution at low pH values[19], the uncharged (more active) form of caffeine being predominant in solution at high pH values. The proportion of uncharged (highly active) to total quinine in solution is also affected by pH, the uncharged portion increasing as the pH is raised[3]. It is possible that enhancement of quinine contracture tension with elevation of pH may be due to greater availability of uncharged quinine in solution at these high pH values. However, this view is difficult to reconcile with the observation that changes in pH had little effect on the duration of the quinine contracture of crab skeletal muscle. In addition, lowering of the pH of crab ringer did not cause any noticeable precipitation of the quinine, an event often observed with caffeine[19,20], even though a quinine concentration of 5 m*M* is near saturation. A pH interaction with the excitation-contraction coupling mechanism would seem to be a more likely reason for pH modification of contracture tension. It is clear that any modification of the quinine interaction with the excitation-contraction coupling mechanism by changing the pH does not involve a change in the main two peripheral parameters of membrane potential and mechanical threshold. Since the speed of pH-induced tension changes of quinine contractures suggests an extracellular site of action, but not at the fibre periphery, the transverse tubular system would seem the most likely site of pH-induced action on the excitation-contraction coupling.

One possibility is that elevation of pH may sensitize the transverse tubular system to any depolarizing action of quinine and potassium, thus accelerating the inward transmission of the surface depolarization and leading to a greater than normal release of calcium from the sarcoplasmic reticulum. This view would explain the increase in the rate of rise of contracture tension with increasing pH value. Alternatively, a high pH value may facilitate a rapid and greater than normal calcium release from the dyadic junctions between the transverse tubular system and the sarcoplasmic reticulum[21], and a low pH may inhibit calcium release. In this case, pH changes would affect the calcium control system of the dyad itself from an extracellular site, but without noticeable effect on the transverse tubules, thus short-circuiting the normal initial stages of the excitation-contraction coupling mechanism. Either view would account for the striking alterations seen in the rising phase of both potassium-induced and quinine-induced contractures as the pH is altered.

Résumé. Les contractures des muscles squelettique du cancre *Carcinus maenas* déclanchées par la quinine sont augmentées si on soulève le pH du milieu extracellulair et se diminue par la réduction du pH. On propose que le lieu d'action du pH sur ces contractures est le système des tubules transversales ou le changement du pH peu modifier la mobilisation du calcium.

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[16] H. C. LUTTGAU and H. OETLIKER, J. Physiol., Lond. *194*, 51 (1968).
[17] H. HUDDART and R. G. ABRAM, J. exp. Zool. *171*, 49 (1969).
[18] D. J. CHIARANDINI, J. P. REUBEN, L. GIRARDIER, G. M. KATZ and H. GRUNDFEST, J. gen. Physiol. *55*, 640 (1970).
[19] G. B. FRANK, S. SING and W. C. BUSS, Archs. int. Pharmacodyn. Thér. *184*, 93 (1970).
[20] H. HUDDART, unpublished observations.
[21] H. HUDDART and K. OATES, Comp. Biochem. Physiol. *36*, 677 (1970).
[22] I am most grateful to Dr. J. WILSON for assistance in preparing this manuscript.

Antihypertensive Effect of Purified Enzyme Showing Angiotensinase Activity from Rabbit Red Cells in Rats

Our previous report has described a purification method and properties of the enzyme showing angiotensinase activity from rabbit red cell hemolysate[1]. The present study was undertaken to observe the effect of s.c. injection of the enzyme on the blood pressure levels in experimental renal hypertensive rats. The experiment was also designed to follow the changes in the levels of plasma renin and angiotensinase activities in acute hypertensive rats.

Materials and methods. The enzyme was prepared from rabbit red cell hemolysate by the fractionation with ammonium sulfate and DEAE cellulose column chromatography[1]. The enzyme, which inactivated completely 300 µg of Val^5-angiotensin-II-amide (Ciba) during 1 h incubation, was injected s.c. in a dose of 5 mg of protein per rat daily. The preparation did not show any depressor effect in rats when injected i.v.

Experiment 1. 17 rats with Goldblatt-type hypertension were divided into the following 2 groups. The experimental 9 rats were injected the enzyme solution (0.5 ml) during 5 weeks period following nephrectomy. The control 8 rats received the injection of phosphate buffer solution (0.1 *M*, pH 6.8). Blood pressure was measured

[1] T. KOKUBU, H. AKUTSU, S. FUJIMOTO, E. UEDA, K. HIWADA and Y. YAMAMURA, Biochim. biophys. Acta *191*, 668 (1969).