females, 19 had no coloured, 4 had slightly coloured and 2 had moderately coloured xanthophores, indicating 8% error. In conclusion, the error in the discrimination of genetic sex in the d-*rR* medaka embryos by the present method is less than 10% [9].

Zusammenfassung. Die Körperfarbe des männlichen d-*rR* Medaka ist wegen des in Xanthophor enthaltenen Carotinoid-Pigments orange, die der weiblichen wegen seiner Abwesenheit uni. Die Färbung erfolgt im allgemeinen nach der Ausbrütung. Wird das rote Carotinoid des Paprikas unmittelbar nach der Befruchtung injiziert, so wird die dorsale Körperoberfläche des männlichen Embryos durch das Pigment gefärbt, was die Feststellung des erblichen Geschlechts schon vor der Ausbrütung erlaubt. Beurteilungsfehler konnten bis auf weniger als 10% herabgedrückt werden.

K. TAKEUCHI

Department of Biology, Aichi-Gakuin University, Nagoya (Japan), 17th February 1967.

[9] The author thanks Prof. T. YAMAMOTO for the use of the facilities in his laboratory and for his kind advice on the work.

Contractility Cycle of an Isolated Gastropod Ventricle[1]

Molluscan and vertebrate cardiac muscle differ in at least one of the properties that are said to make discussion of mechanical properties of the latter difficult, if couched in the terms developed for skeletal muscle. Those properties of vertebrate cardiac muscle are: (1) presence of 'appreciable resting tension...at all lengths at which active tension is developed'[2]; (2) marked dependence of isometric tension on length throughout the length-tension diagram[2-4]; (3) 'time dependence of onset of active state'[5] and (4) that normal cardiac muscle cannot be tetanized and therefore cannot provide a measure of maximum contractility independent of time[3].

It has been known since the last century that molluscan cardiac muscle can be tetanized[6]. If it were established that molluscan and vertebrate myocardium were alike in property (3), molluscan cardiac muscle would offer a means of studying a contractile tissue with slow onset of 'active state' in terms meaningful with regard to the body of work on skeletal muscle, for which the onset of active state is abrupt[7].

The method of following the course of activation of contractility by quick release[8] has been applied by BLEICHERT et al.[9] to spontaneously beating rings made from the ventricle of the heart of a species of *Aplysia* (Opisthobranchia Anaspidea). Their results may be interpreted as showing that active state increased during the first third of an isometric single contraction, remained constant until shortly before peak tension, fell sharply at the peak, and shortly thereafter became zero. Their finding that at least in *Aplysia* (and presumably in other anaspid opisthobranchs) the heart muscle is extraordinarily plastic, developing the same tension over a 10 times change in length, suggests property (2) is not so marked for mollusc hearts. However, the mollusca possess great diversity in cardiac architecture and consequent strength of the ventricular wall[6]. This report deals with the time course of active state, as determined by quick stretch and quick release techniques, in the isolated perfused ventricle of a mollusc with a robust heart, *Busycon canaliculatum* (Prosobranchia).

Methods. Before each experiment the ventricle in its bath was stretched vertically between aorta and auricle to a chosen initial tension such that fibers in trabeculae oriented in that axis[10] would contract in essentially isometric fashion, although the ventricle could beat spontaneously since the contraction of circularly oriented fibers was essentially isotonic. Spontaneous beating was maintained by perfusion with aerated sea water at a head of 60 cm H_2O through a cannula in the auricle. Quick stretches and releases were given by a Levin-Wyman ergometer adapted so that tension was detected by a RCA 5734 mechano-electronic transducer mounted at the end of the moving beam of the ergometer. The plate shaft of the transducer was connected by a nylon thread to the aorta of the *Busycon* ventricle in such a way that the aorta was not occluded. Movement of the ergometer beam was detected by the use of an Ether Ltd. PD 20 displacement transducer, and output of both transducers was displayed simultaneously on a dual beam cathode ray oscilloscope. All experiments were performed during June, July, and August of 1966 at room temperature of 23–29 °C. 42 preparations were used.

Results. Figure 1 is a photographic record of a representative series of quick (5 msec) releases to zero tension. These 4 releases were chosen to represent releases at half-systole, two-thirds systole, full systole and one-third diastole from among 46 quick releases of 0.75 mm given to one ventricle at 25 °C. Redevelopment of tension is maximal following release early in the cardiac cycle (Figure 2 b) but the capacity to redevelop tension persists until quite late in diastole (Figure 2 e). If a release is given during diastolic pause, the next contraction develops the lower tension characteristic of the shorter length (Figure 2 a). Thus giving a quick release during a beat shifts the regenerated beat to the tension curve characteristic of the new length; the amount of tension redeveloped being dependent on how much of the time course of the beat is still to run. Similarly, after a quick stretch we find that the isometric contraction proceeds along its original time

[1] Research supported by National Science Foundation grant No. GB-1001.

[2] A. J. BRADY, J. Physiol. *184*, 560 (1966).
[3] A. J. BRADY, Fedn Proc. Fedn Am. Socs exp. Biol. *24*, 1410 (1965).
[4] V. J. FISHER, R. J. LEE, A. GOURIN, H. BOLOOKI, J. H. STUCKEY and F. KAVALER, Am. J. Physiol. *211*, 310 (1966).
[5] E. H. SONNENBLICK, Fedn Proc. Fedn Am. Socs exp. Biol. *24*, 1396 (1965).
[6] R. B. HILL and J. H. WELSH, *Physiology of Mollusca*, (Ed. K. WILBUR and C. M. YONGE; Academic Press, New York 1966), vol. 2, p. 125.
[7] A. V. HILL, Proc. R. Soc. B, *136*, 399 (1949); *137*, 320 (1950); *138*, 339 (1951); *141*, 498 (1953).
[8] H. S. GASSER and A. V. HILL, Proc. R. Soc. B, *96*, 398 (1924) A. V. HILL, Proc. R. Soc. B, *100*, 108 (1926).
[9] A. BLEICHERT, U. HAGEMEIR, H. REICHEL and E. SIEHR, Pubbl. Staz. zool. Napoli, *34*, 317 (1965).
[10] R. BRUNET and A. JULLIEN, Archs Zool. exp. gén. *78*, 375 (1937).

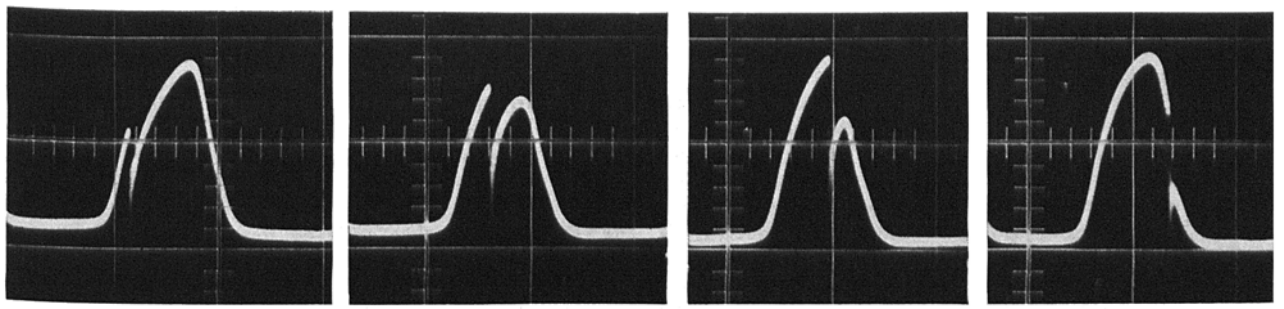

Fig. 1. Ordinate: tension, 0.105 g/small division. Abscissa: time, 4 sec/large division.

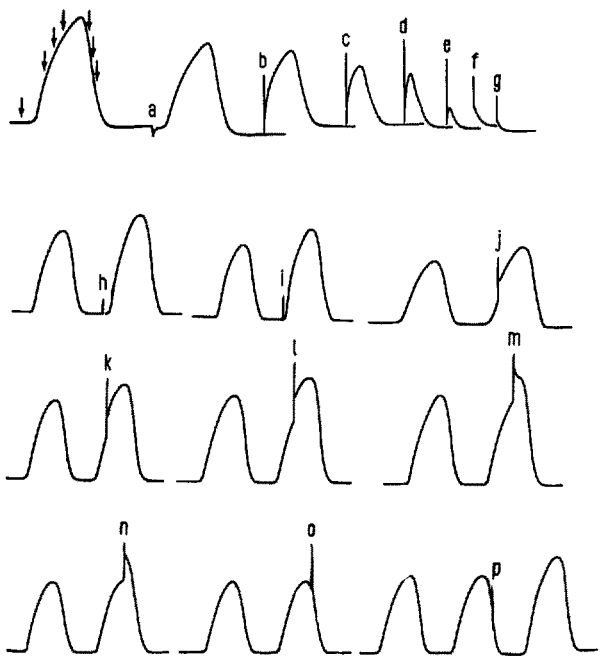

Fig. 2. Tracings of spontaneous isometric contractions recorded as in Figure 1. At points a–g the ventricle was released and allowed to shorten 0.75 mm. At points h–p the ventricle was stretched 0.75 mm.

course, but on the tension development curve characteristic of the greater length. In Figure 2, h–p are the tracings of a representative series of 9 quick (5 msec) stretches selected from among 27 stretches of 0.75 mm given to 1 ventricle at 29 °C. A quick stretch in diastolic pause (Figure 2 h) leads to the tension development characteristic of the greater length in the next contraction, although the stretch itself revealed only the low degree of stiffness to be found at any point in the diastolic pause. A stretch just before initiation of systole (Figure 2 i) reveals greater stiffness, but the stiffness continues to increase as the ventricle contracts, reaching a maximum around peak systole (Figure 2 m, n and o). Stretch applied after peak systole does not produce the peak tension characteristic of the new length until the next cycle (Figure 2 p) even if applied while stiffness of the contracted ventricle is still high (Figure 2 o). Thus molluscan cardiac muscle differs markedly in its response to quick stretch from vertebrate skeletal muscle, where decreased extensibility develops much more rapidly than does isometric tension[7].

Discussion. Similar results have been reported for isovolumetric tortoise ventricle[11], and for rabbit and cat papillary muscle[2,3]. In addition, the method of taking instantaneous velocity of shortening following quick release as a measure of active state[5] revealed that in cat papillary muscle the time course of active state is slow, both in development and in decay; as it is in *Busycon* ventricle. Thus although molluscan cardiac muscle is quite different in histological structure from vertebrate cardiac muscle[6] and differs in physiological properties at least to the extent of being tetanizable, it shares the property of slow onset and decay of contractility.

It might well be asked what cardiac muscle of the 2 groups has in common that leads to the typically slow time course of cardiac active state in both. One property possessed by ventricular cardiac muscle of both that sets it apart from skeletal muscle is the action potential with a plateau[12–14]. That fact may be relevant if the generation of active state is dependent on the 'mechanically effective period' of the action potential; a hypothesis proposed by SANDOW[15] and supported by the observation that maximum tension attained in a single beat is greatly diminished when the plateau potential of molluscan ventricular myocardium is abolished (but the spike retained), either by sudden release[14] or with acetylcholine[12]. If 'activator' is caused to enter heart muscle fibers during the action potential it might well be that slow development of active state in cardiac muscle is due to slow inward diffusion of activator and that slow decay of active state is due to long persistence of the mechanically effective period of the action potential[16].

Zusammenfassung. Plötzliche Dehnungen oder Entspannungen während des Eigenrhythmus der Herzkammer von *Busycon canaliculatum* wurden dazu benützt, um den Verlauf des Aktivitätszustandes zu untersuchen. Sowohl Anstieg, wie auch Abfall des «active state» waren entsprechend langsam wie bei Wirbeltier-Herzmuskeln. Das tetanisierbare Schneckenherz stellt somit ein gutes Versuchsobjekt für die Aktivierung des Herzmuskels dar, da die maximale Kraft unabhängig von der Zeit ist.

R. B. HILL and P. J. SCHUNKE

Department of Physiology, Dartmouth Medical School, Hanover (New Hampshire 03755, USA), 20th January 1967.

[11] L. J. O'BRIEN and J. W. REMINGTON, Am. J. Physiol. *211*, 770 (1966).
[12] H. IRISAWA, M. KOBAYASHI and T. MATSUBAYASHI, Jap. J. Physiol. *11*, 162 (1961).
[13] A. EBARA, Jap. J. Physiol. *16*, 371 (1966).
[14] H. NOMURA, Sci. Rep. Tokyo Kyoiku Daig. B, *11*, 153 (1963).
[15] A. SANDOW, S. R. TAYLOR and H. PREISER, Fedn Proc. Fedn Am. Socs exp. Biol. *24*, 1116 (1965).
[16] W. F. H. M. MOMMAERTS, B. C. ABBOTT and W. J. WHALEN, *Structure and Function of Muscle* (Ed. G. H. BOURNE; Academic Press, New York 1960), vol. 2, p. 517.