varying in amplitude through this stage of 26 sec duration (Figure B). Successively, the spikes reached the maximum in size and a regular frequency within 15 sec, while the animal appeared catatonic (Figure C). Within 6 sec these rhythmic frequent spike discharges were retarded and depressed (Figure D). The cessation of the convulsion stage was followed by silence of all the electrical activities. After 20 sec small spikes were recognized again at the low frequency of 1 every 1–2 sec. The animal manifested stupor sometimes with a characteristic posture like a kangaroo and adversive movements of the head to the left (Figure E). Oral movements or resuming behavior were present at times simultaneously with the arousal pattern of the EEG for a brief duration (Figure F). This post-ictal stage continued for 6 min and recovery both in the EEG and behavior was seen. In the records of the El mouse, the paroxysmal discharges were mostly composed of spikes of duration within 120 msec with fewer slow waves.

In the inter-ictal period also paroxysmal discharges could be observed less frequently and in limited regions. Even during drowsiness, deep sleep or the REM sleep, the spikes occurred at the same regions with a short duration. In abortive seizures, which were only squeaking and/or an instantaneous catatonic posture and were often seen at the first few weeks after the commencement of the seizures, repetitive spike discharges occurred in same regions but were unlikely to spread and generalize.

The present paper reports for the first time the EEG of the hereditary epileptic mouse while freely moving. On the audiogenic seizure mouse, there was the EEG recording for a short duration only under anesthesia[6]. An El mouse is considered as a kind of the sensory precipitated epilepsy[7] comparable to similar types of epilepsy in man, a mouse with the audiogenic seizure[8], a baboon of photic sensitivity[9], a particular strain of a Mongolian Gerbil[10] and a domestic fowl[11], because of its susceptibility to changing position or environment. The paroxysmal discharges have a certain localized onset at the centro-parietal region of the hemisphere and spread to become general over the whole cortex and are followed by sudden depression in the El mouse. During the seizure, the features of the paroxysms are quite similar to those in a photogenic baboon, except for the focus. In the latter, the spikes started at the fronto-central region[9].

As the provoking conditions of seizures in El mice are rather complicated and obscure in nature, investigations on them should be made. So far the seizures seem likely to be caused by vestibular or proprioceptive stimulations.

[6] R. N. HARNER, Electroenceph. clin. Neurophysiol. *13*, 752 (1961).
[7] R. G. BICKFORD and D. W. KLASS, in *Basic Mechanisms of the Epilepsies* (Eds. H. H. JASPER, A. A. WARD, JR. and A. POPE; Little, Brown, Boston 1969), p. 543.
[8] R. L. COLLINS, in *Experimental Models of Epilepsy* (Eds. D. P. PURPURA, J. K. PENRY, D. TOWER, D. M. WOODBURY and R. WALTER; Raven Press, New York 1972), p. 347.
[9] R. NAQUET and B. S. MELDRUM, in *Experimental Models of Epilepsy* (Eds. D. P. PUPURA, J. K. PENRY, D. TOWER, D. M. WOODBURY and R. WALTER; Raven Press, New York 1972), p. 373.
[10] W. J. LOSKOTA and P. LOMAX, Electroenceph. clin. Neurophysiol. *38*, 597 (1975).
[11] E. C. CRICHLOW and R. D. CRAWFORD, Can. J. Physiol. Pharmac. *52*, 424 (1974).

Preparation of Isolated Single Cardiac Cells from Adult Frog Atrial Tissue[1]

M. TARR and J. W. TRANK[2]

Department of Physiology, University of Kansas Medical Center, College of Health Sciences and Hospital Kansas City (Kansas 66103, USA), 17 September 1975.

Summary. Isolated cardiac cells from bullfrog atrial tissue can be readily prepared by digestion of intact fragments of atrial tissue with trypsin and collagenase. These isolated cells have dimensions of about 5 μm in width and range in length from 300 μm to over 500 μm. Such isolated cells may prove useful for the investigation of contractile activity of cardiac muscle at the single cell level and at the sarcomere level within the single cell.

The preparation of component cells from various tissues perfusion with a variety of proteolytic enzymes has been known for some time. However, the preparation of isolated cardiac cells has been primarily confined to the embryonic chick heart. Only recently have attempts been made to isolate viable cardiac cells from adult myocardial tissue and to date the majority of this work has been confined to mammalian cardiac muscle.

In 1970 VAHOUNY et al.[3] described a method for the preparation of isolated cardiac cells from adult male rats by incubation of the heart fragments in a saline solution containing trypsin and collagenase. Isolated cells obtained by this method were spontaneously active and remained active for several hours. Since then, several investigators have used VAHOUNY's technique to prepare single smooth muscle cells from the stomach muscle of *Bufo marinus*[4,5]. In the present study, we adapted the technique for the preparation of isolated cardiac cells from adult bullfrog atrial muscle.

Materials and methods. Bullfrog (*Rana catesbeiana*) atrial tissue was cleaned of as much noncardiac cell tissue as possible and then minced into coarse fragments. The fragments from one atrium were placed in 2 ml of a Ca^{++}-free Ringer's digestion solution having the following composition: 111 m*M* NaCl, 5.4 m*M* KCl, 10 m*M* *tris*

[1] This investigation was supported by U.S. Public Health Service, National Institutes of Health Grant No. HL 12426 and a Kansas Heart Association Grant-in-Aid.
[2] We wish to thank Dr. J. SOMMERS of Duke University Medical Center for doing electron microscopy of the isolated cells to confirm the single cell nature of the preparations.
[3] G. V. VAHOUNY, R. WEI, R. STARKWEATHER and C. DAVIS, Science *167*, 1616 (1970).
[4] R. M. BAGBY, A. M. YOUNG, R. S. DOTSON, B. A. FISHER and K. MCKINNON, Nature, Lond. *234*, 351 (1971).
[5] F. S. FAY and C. M. DELISE, Proc. natn. Acad. Sci., USA *70*, 641 (1973).

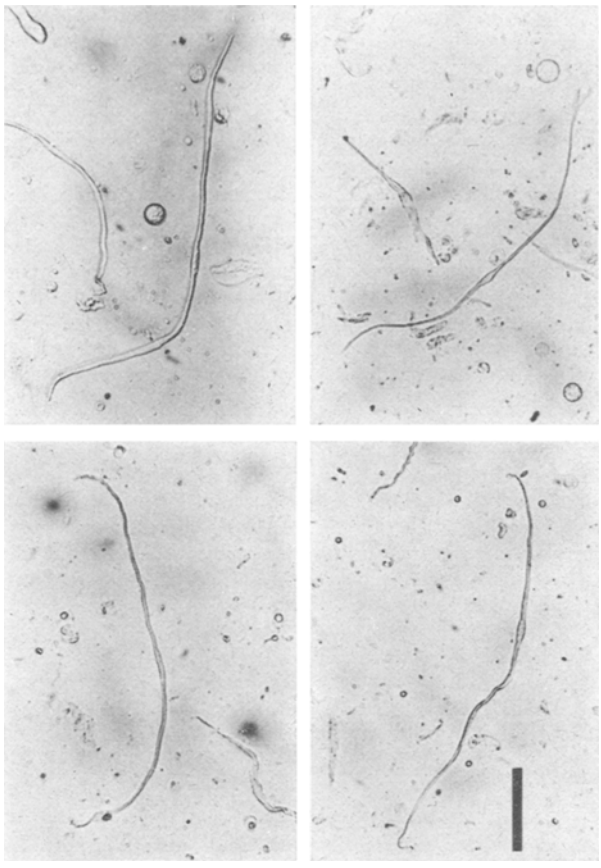

Fig. 1. Examples of 4 typical single frog atrial cells prepared by trypsincollagenase dispersion of intact frog (*Rana catesbeiana*) atrial tissue; length calibration is 100 μm.

(hydroxymethyl) aminomethane, 0.1% collagenase, and 0.1% trypsin. The pH of this Ca^{++}-free digestion solution was adjusted to 7.0 at 25 °C by adding 12.4 *N* HCl. The digestion medium with tissue fragments was agitated by gentle bubbling with 95% O_2–5% CO_2 and at 30 min intervals the supernatant was removed and replaced by 2 ml of fresh digestion solution and the digestion continued. The supernatant from each 30 min digestion period was added to 10 ml of cold Ca^{++}-free Ringer's solution (composition as above without trypsin and collagenase) and the heart cells were collected by gentle centrifugation. All procedures were carried out at room temperature. The cells from each 30 min harvest were resuspended in 6–10 ml of *Tris*-buffered Ringer's solution containing 1.8 m*M* $CaCl_2$, 4 m*M* glucose, 100 U penicillin/ml, and 200 μg streptomycin/ml. Cells contained in 1–2 ml of this solution were plated into tissue culture dishes (Falcon tissue culture dish No. 3001) and allowed to settle and attach to the bottom of the dish. The cells attach firmly enough to the culture dishes so that the cells can be superfused with solution of varying composition. It was found that a significant number of cardiac cells was obtained only after $1^1/_2$–2 h of tissue digestion, a result similar to that reported by VAHOUNY et al.[3]. No obvious structural differences were observed between cells obtained 2 h after initiation of digestion and those obtained 4 h after initiation of digestion.

Results. A large quantity of isolated cells can be obtained by this technique and examples of 4 typical cells are shown in Figure 1. These cells are spindle shaped and have a centrally located nucleus; bifurcated cells have also been observed. The cells are quite long ranging in length from about 300 μm to over 500 μm. The cell width at the region of the nucleus is on the order of 9 μm, whereas adjacent to the nucleus region the cell width is about 5 μm. We have verified by hemotoxylin-eosin staining of the isolated cells and by electron microscopy

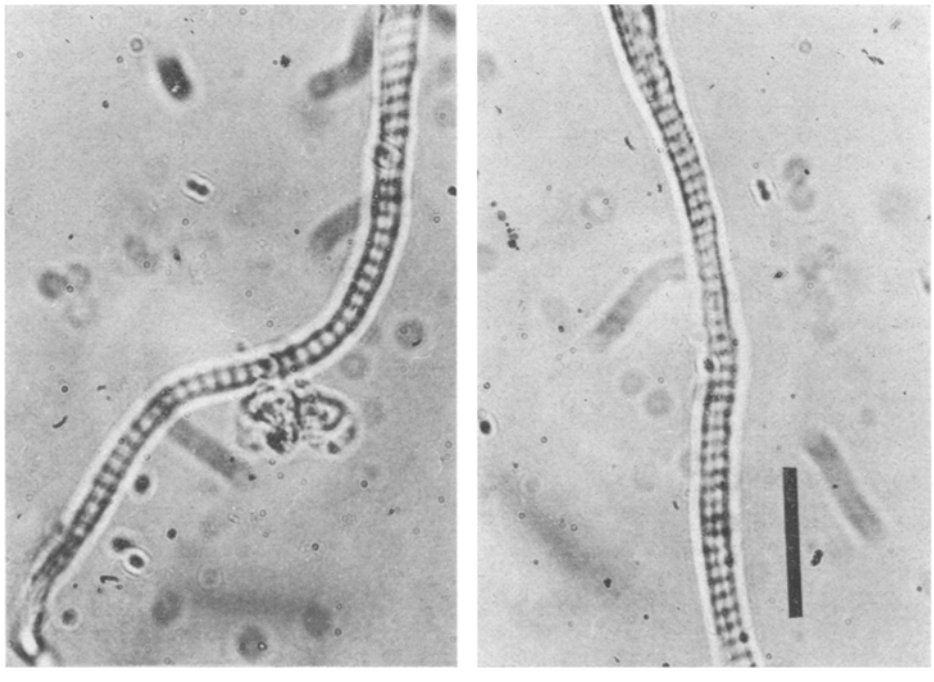

Fig. 2. Examples of sarcomere pattern in 2 cells observed at high magnification; length calibration is 20 μm.

that the cells prepared by this technique are single cells.

Observation of the cells at high magnification (Figure 2) demonstrates that the cells have a distinct sarcomere patterning and that the sarcomere spacing is fairly uniform along the length of the cell. The sarcomere spacing (determined by dividing the number of sarcomeres in a given region of the cell by the length of that region) is about 2.1 μm (average of 11 determinations on 10 cells).

A number of preliminary tests have been performed to assess the viability of these isolated cells. The ability of these cells to give twitch like contractions in response to an electrical stimulus was determined. Electrical stimulation was provided by field stimulation between an electrode placed in close proximity to a cell and a remote electrode placed in the fluid in the culture dish. Of 479 cells (from 7 atria) tested within 5 h of preparation, 380 (79%) of the cells gave twitch like contractions in response to electrical stimulation. The response of the isolated cells to elevated extracellular potassium concentration was also determined. Of 101 cells (from 5 atria) superfused with high-K Ringer's solution (115 m*M* KCl, 111 m*M* NaCl and 1.8 m*M* $CaCl_2$), 87 (89%) of the cell produced either sustained or transient contractile responses. Very few cells showed spontaneous contractile activity when exposed to normal Ringer's solution.

Discussion. The technique of trypsin and collagenase dispersion of intact tissue appears to work nicely for the preparation of isolated frog atrial cells. The morphology of cells obtained by this technique appears to be similar to that obtained by MARCEAU[6] on cells isolated from frog cardiac tissue by a combination of potassium caustique and acid dissociation of intact tissue. The cell width of 5 μm is similar to that obtained by MARCEAU as well as that reported by electron microscopists[7]. The cell length of 300–500 μm is longer than reported previously in the literature. BARR et al.[8] reported cell lengths of 175 ot 250 μm on frog atrial cells teased from bundles incubated in EDTA Ringer's and SKRAMLIK[9] reported lengths of 73 to 193 μm in cells isolated by KOH digestion of intact tissue. However, the diagrams of frog cardiac cells presented by MARCEAU[6] give a length to width ratio of about 70 indicating cell lengths in excess of 300 μm. The sarcomere spacing in the isolated cell of about 2.1 μm is similar to that reported for intact frog cardiac tissue[10,11].

The isolated cells prepared from adult bullfrog atrial tissue appear to have intact membranes as evidenced by the observations that they give contractile responses in response to electrical stimulation or an elevation in extracellular potassium concentration. In contrast to mammalian cardiac cells prepared by enzymatic digestion where only about 10% of the cells appear to have intact membranes[12], the majority of the isolated frog atrial cells appear to have normal morphology and respond to electrical or chemical stimulation. Also, these frog atrial cells tolerate extracellular calcium concentrations in excess of 1 m*M*, whereas isolated mammalian cardiac cells go into contracture if extracellular calcium concentration is elevated above 1 m*M*[12].

[6] F. MARCEAU, Annls Sci. nat., Zoologie *19*, 191 (1904).
[7] N. STALEY and E. BENSON, J. Cell Biol. *38*, 99 (1968).
[8] L. BARR, M. M. DEWEY and W. BERGER, J. gen. Physiol. *48*, 797 (1965).
[9] E. VON SKRAMLIK, Z. ges. exp. Med. *14*, 246 (1921).
[10] R. NASSAR, A. MANRING and E. A. JOHNSON, in *The Physiological Basis of Starling's Law of the Heart* (Ciba Foundation Symposium 24, Ass. Scientific Publishers, New York 1974), p. 57.
[11] S. WINEGRAD, J. gen. Physiol. *64*, 343 (1974).
[12] A. FABIATO and F. FABIATO, Circulation Res. *31*, 293 (1972).

A Population of Human Lymphocytes Staining for Esterases[1]

N.R. FARID, E.P. NOEL and C. KUTAS

Faculty of Medicine, Memorial University of Newfoundland, St. John's (Newfoundland, Canada A1C 5S7), 17 September 1975.

Summary. A population of lymphocytes is found to stain positively for esterases. The positively staining lymphocytes are more predominant among T than B lymphocytes and are significantly increased on stimulation with PHA. Treatment with cholinestrase inhibitors reduces their number significantly.

Staining for non-specific esterase is a widely used cytochemical method for the identification of monocytes. However, it has not been reported that lymphocytes stain positively using this technique. We now report that, in contrast to the diffuse cytoplasmic staining pattern observed with monocytes, a variable proportion of lymphocytes show one or more discrete cytoplasmic 'spots'. We examined the possibility that positively staining lymphocytes may correlate with distinct subpopulations of lymphocytes. We also studied the effect of lymphocyte stimulation with phytohaemagglutinin (PHA).

Blood leukocytes from 26 persons were separated by Hypaque-Ficoll gradient centrifugation[2]. The average yield of lymphocytes was 85% and 95% of the cells were lymphocytes. Viability as judged by dye exclusion was 99%. Sheep red blood cell (SRBC) rosette formation with T lymphocytes was carried out as previously described[3]. Rosette forming lymphocytes were separated from non-rosetting lymphocytes by gradient centrifugation[4]. The sedimented rosettes and the free cells harvested from the interface were used as source of T and B-rich leukocytes suspensions respectively. Adherent cells were depleted by incubating 8×10^6 leukocytes in 2 ml of medium in small petri dishes at 37 °C for 2 h[5].

Lymphocyte proliferation was induced by culturing 4×10^6 cells/ml with 12.5 μl of PHA (Burrough-Wellcome

[1] This work was supported by a grant from the Medical Research Council of Canada.
[2] A. BOYUM, Scand. J. clin. Lab. Invest. *21*, Suppl. 97 (1968).
[3] M. JONDAL, G. HOLM and H. WIGZELL, J. exp. Med *136*, 207 (1972).
[4] M. F. GREAVES and G. BROWN, J. Immun. *112*, 420 (1974).
[5] G. KRIKORIAN, W. H. MARSHALL, S. SIMMONS and F. STRATTON, Cell Immun. *19*, 22 (1975).