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].

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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

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Proportion of esterase positive lymphocytes (EPLs)

Untreated lymphocyte suspension	Adherent cell depleted	SRBC rosette forming lymphocytes[a]	Free cells (B-rich fraction)	Following PHA stimulation
12.8 ± 6.1 (26)[b]	14.0 ± 6.1 (8) (NS)	17.7 ± 8.6 (12) ($p < 0.05$)	11.7 ± 4.3 (12) (NS)	17.8 ± 6.3 (12) ($p < 0.03$)

[a] The SRBC rosettes formed a mean (± SD) of 65 ± 5.6% of the untreated lymphocyte suspension. [b] A minimum of 200 lymphocytes or rosettes were counted and the proportion of EPL calculated. Number in brackets signify number of observations. NS, not significant.

batch K6674) for 72 h. Parallel cultures were pulsed with ^{3}H-thymidine at that time to ensure that proliferation has occurred. Cell viability at the end of 72 h was greater than 85%.

Smears prepared from lymphocytes or rosettes were fixed in 25% formaldehyde and stained for non-specific esterase[6].

The Table shows the results obtained. The T lymphocyte fraction shows a higher proportion of esterase positive lymphocytes (EPL) than either the B lymphocyte fraction or unfractionated lymphocytes. This observation was directly confirmed when smears of rosette preparations were made, and it was found that cells forming rosettes had a higher percentage of EPLs than unfractionated lymphocytes. Culture with PHA also increases the proportion of EPLs. Furthermore PHA stimulation causes some lymphocytes to acquire a rim-staining pattern.

Our results make it unlikely that the mononuclear cells showing discrete staining for non-specific esterase are monocytes with lymphocyte-like morphology. The proportion of EPLs remains unchanged following depletion of adherent cells, and following such treatment there tends to be a greater number of EPLs which form SRBC rosettes. Monocytes do not form rosettes with untreated SRBC[7].

While accumulating evidence points to the fact that PHA induces proliferation of both T and B lymphocytes as measured by ^{3}H-thymidine uptake in the human[8–10], work by GREAVES et al.[11] and JONDAL[12] indicate that the overwhelming majority of blast cells developing in response to PHA carry surface markers specific to T lymphocytes. We have studied the effect of PHA-induced proliferation to investigate the possibility that the appearance of esterase staining is associated with an increase in lymphocyte metabolic activity. Our findings of PHA-induced increase in EPLs is in keeping with the previously reported observation that immunoflorescent staining for cholinestrase increases following PHA stimulation[13].

It is significant that esterase activity is represented more on cells of the T lymphocyte differentiation axis, in view of the recent observations that anticholinestrase agents inhibit SRBC rosette formation[14]. The effect of these pharmacologic agents on B lymphocyte receptors is still unknown. Our preliminary results show that cholinestrase inhibition by physostigmine sulphate and depletion by echothiophate iodide significantly reduces the proportion of EPLs[15]. The biological significance of the presence of cholinestrase activity on lymphocyte membranes is not established; however it has been suggested that it may play an essential role in the stabilization of membrane structure[14] and in the process of lymphocyte activation[13].

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Invertase Activity in the Midgut of *Sarcophaga ruficornis* and *Musca domestica* (Diptera: Insecta)

M. SINHA

Department of Zoology, University of Lucknow, Lucknow (India), 17 July 1975.

Summary. The pH for the optimum activity of the midgut invertase was 5.5 in the adults of *S. ruficornis*, 6.0 in its larvae and adults of *M. domestica* and 6.5 in the larvae of the latter fly. The optimum temperature was 50 °C. Enzyme activity was retarded by the addition of glucose and fructose.

Adults of *S. ruficornis* and *M. domestica* survived well on cane-sugar and water, while their larval stages failed to develop on this diet inspite of the fact that both the stages possess the enzyme required for the hydrolysis of cane-sugar, namely, invertase (unpublished observations). Presuming that there might be some difference in the nature of the enzyme of the two stages, the effect of various factors on the invertase activity has been studied.

Materials and methods. S. ruficornis was reared on sugar and meat, and *M. domestica* on sugar and milk[1]. Midgut homogenate was prepared as described by SINHA[2]. The enzyme concentration was 4 guts/ml and $^1/_2$ gut/ml in the case of larvae and adults respectively.

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