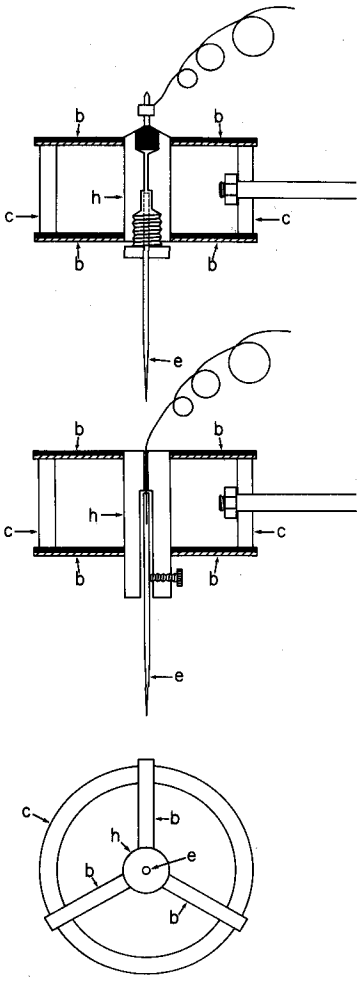

Diagram of the piezoelectric vibrator in side and top view. *b* piezoelectric beams, *c* lucite housing, *d* glass microelectrode, *e* holder, where glass microelectrode is fastened.

unidirectional displacement of all elements. Polarization was checked by the measuring of the evoked potential in the piezoelectric crystal, after its mechanical distortion. (One end of the crystal was clamped and opposite side manually displaced.) The conductive silver epoxy cement (Epoxy Products Co., Div. of Allied Products Corp.) was used for fastening of wires to the piezoelectric elements. In our experiments, the beams were excited by 3-10 V of 2 or 4 kHz by audio oscillator. These parameters were chosen according to the calibration curve, when whole system, including inserted microelectrode was calibrated with an electro optic sensor (Mechanical Technology Corp.). The electro optic sensor is emitting light through the fine optic fibres and reflected light from observed subject is perceived through other part of the same probe.

In an attempt to establish an electrical potential profile during the glass microelectrode penetration, the microdrive of the manual manipulator was mechanically coupled with a brass ring to a multiturn potentiometer and output of potentiometer connected to an X-Y plotter. Applied voltage and size of the potentiometer was selected according to the sensitivity or range of X-Y plotter. (In our case 15 V from power supply, 10 kΩ 10 turn potentiometer, Hewlett-Packard X-Y plotter.) The distance of microelectrode introduction was displayed on the X-axis and the size of the measured potential was recorded on the Y-axis. Described microelectrode vibrator is applicable in any situation where the penetration of the membranes without their displacement is necessary.

1 This study was supported by the University Research Council, UNC, Chapel Hill, North Carolina. Vernitron Piezoelectric Division, Bedford, Ohio, generously supplied piezoelectric beams.
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Ischemic myocardial injury in cultured heart cells: In situ lysosomal damage[1]

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Summary. Primary cultures of rat myocardial cells which were subjected to oxygen and glucose deprivation, 2 conditions associated with ischemia, were evaluated for alterations in lysosomal integrity. A photometric technique measured changes in latent acid phosphatase activity and lysosomal membrane permeability.

Because lysosomal alterations have been implicated to occur during myocardial ischemia[2-4], a lysosomal concept of myocardial injury has evolved in which ischemia is thought to labilize lysosomal membranes, resulting in release of potent hydrolytic enzymes into the cytosol and subsequent damage to important cellular components. However, a critical question that has to be answered is whether lysosomal alterations precede and initiate ischemic injury or whether lysosomal changes result as a consequence of the events that produce the injury or necrosis. To explore this problem, we have developed an in vitro cellular model of ischemic injury with primary cultures of rat myocardial cells[5,6]. The effect of in vitro ischemia on in situ, unfixed lysosomes of myocardial cell cultures was evaluated by a photometric method[7] which gives a quantitative measurement of lysosomal membrane permeability and latent acid phosphatase activity.

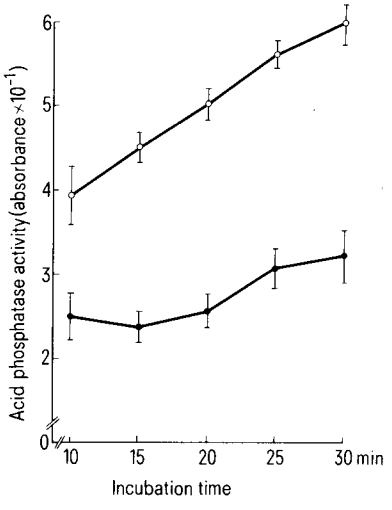
Materials and methods. Primary cultures of rat myocardial cells were isolated and grown by the method of Wenzel et al.[8]. The cultures were grown to confluence on coverglasses in plastic petri dishes with Eagle's minimum essential medium (MEM) and 5% fetal calf serum. Myocardial 'ischemia' was simulated in vitro by subjecting the cultures to oxygen and glucose deprivation as developed by our laboratory[5,6]. Control cultures were exposed to 20% O_2 and 1000 mg of glucose/l of medium, while ischemic cultures were deprived of O_2 and glucose for 12 h.

After the treatments, the coverglasses with attached cells were removed from the petri dishes and were incubated for various times in fresh lysosomal cytochemical medium[9,10]. Acid phosphatase activity is demonstrated by the formation of lead phosphate which is subsequently converted to black lead sulfide deposits, highly visible microscopically. Because of the relative impermeability of intact, undamaged lysosomes to β-glycerophosphate, a substrate for acid phosphatase, the greater formation of lead sulfide in

treated cells than in controls is evidence of increased substrate accessibility to acid phosphatase and suggestive evidence of injury to the organelle membranes.

Lead sulfide (as an indicator of acid phosphatase activity) was quantiatively measured by a modification of a photometric method first described by Aikman and Wills[7]. By viewing a monolayer of cells with dark-field microscopy, the amount of light transmitted by the lead precipitates in the cells was measured with a sensitive photometer (Science and Mechanics, Model 102) attached to the microscope. The readings were converted to absorbance by the method of Bitensky et al.[11]. The mean of 5 absorbance readings±SE for each incubation period was plotted against time of incubation in the cytochemical reaction mixture.

Results and discussion. As illustrated by the figure, acid phosphatase activity increased as a function of incubation time. However, acid phosphatase activity of 'ischemic' cultures was significantly higher than that of control cultures ($p < 0.05$, Student's t-test). This study suggests that lysosomal membrane permeability was increased in ischemic cultures which resulted in diminished acid phosphatase latency and greater formation of the reaction product, lead sulfide.



Acid phosphatase activity in primary cultures of rat myocardial cells after treatment with 12 h of oxygen and glucose deprivation. Controls (●); treated cultures (○). After treatment with 'ischemia', the cultures were incubated for designated periods in the lysosomal cytochemical medium. Mean of 5 replicate cultures per point. Vertical bars represent SE.

Although true ischemia may not be reproduced in cultured cells, we have demonstrated that simulated ischemia of oxygen and glucose deprivation in cultured heart cells resulted in a time-related inhibition of beating activity and leakage of cytoplasmic enzymes from the cells into the culture medium[5,6], as well as enhanced lysosomal membrane fragility reported in this study. Thus, 2 important elements of ischemia, transient anoxia and substrate deprivation, have been shown to affect cultured heart cells in a manner qualitatively similar to some of the in vivo effects of myocardial ischemia[4,12].

Finally, the photometric procedure described in this paper is presented as a useful and simple method for quantitating permeability changes of in situ, unfixed lysosomes in cultured cells exposed to injurious conditions. This procedure utilizes inexpensive and readily available instruments such as a darkfield microscope and photometer and thus alleviates the need for the more expensive and sophisticated instrument commonly used in quantiative histochemistry, e.g., microdensitometer.

1 Acknowledgments: The authors thank Dora Fernandez for skillful technical assistance. Research was supported by a grant from the National Heart, Lung, and Blood Institute, HL 18647.

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