Contractile Response of Halothane-Depressed Isolated Atria to Pyruvate

Inhalation anesthetic halothane is widely used in operating rooms. There is evidence, however, that halothane depresses the cardiac function[1,2]. Although the effects of halothane on contractility of the heart have been studied with isolated heart preparations[3–6], few investigations of halothane on metabolism have been made[7,8]. It was of interest to ascertain whether the myocardial depression due to halothane is related to the metabolic behavior as well as the substrate utilization of the myocardium. In the present investigation, it was found that the addition of metabolic substrate, pyruvate, to the halothane-depressed isolated rat atria resulted in a marked increase in the force of contraction.

Methods. Atria from decapitated rats were suspended in a modified Krebs-Ringer bicarbonate glucose medium aerated with 95% O_2-5% CO_2 at 30 °C and pH 7.4, and electrically stimulated at a rate of 200/min as previously described[9,10]. In the experiments with hypertonic medium the increase of osmotic pressure was made by the addition of sodium chloride at a concentration of 100 m*M* to the normal Krebs-Ringer bicarbonate glucose medium. Halothane was administered into the medium by means of the anesthetistat[11]. The halothane concentration in the medium was determined at 10 to 30 min intervals with a gas chromatograph throughout the experimental period[11,12].

Results. Effect of pyruvate on halothane-depressed rat atria. In Figure 1, it was first determined that approximately 6 mg/100 ml halothane was required to maintain a 50% depression of the contractility of rat atria suspended in a modified Krebs-Ringer bicarbonate glucose medium. Sodium pyruvate (5 m*M* and 2.5 m*M*) was added to the bathing medium 30 min after the start of the halothane administration. Despite the continued administration of halothane and maintenance of halothane levels in medium similar to the control atria, the addition of pyruvate resulted in a gradual increase in the force of contraction (Figure 1). The maximally effective concentration of pyruvate was 2.5 m*M*. As a control experiment, addition of pyruvate to normal atria resulted in only slight alterations in contractile activity in contrast to the marked positive inotropic effects seen when administered to halothane-depressed atria (Figure 1).

Effect of NaCl on halothane-depressed rat atria. Experiments were performed to elucidate the nature of pyruvate effect on halothane-depressed atria, in which sodium chloride was employed under the same conditions as pyruvate. Figure 3 shows that 5 m*M* sodium chloride is ineffective in restoring the force of contraction of halothane-depressed atria when administered under the same conditions where sodium pyruvate is effective. This indicates that the effect of sodium pyruvate is due to pyruvate itself.

Effect of pyruvate on atria depressed in hypertonic medium. Experiments to further clarify the pyruvate effect on halothane-depressed atria were designed employing different experimental conditions. Hypertonic

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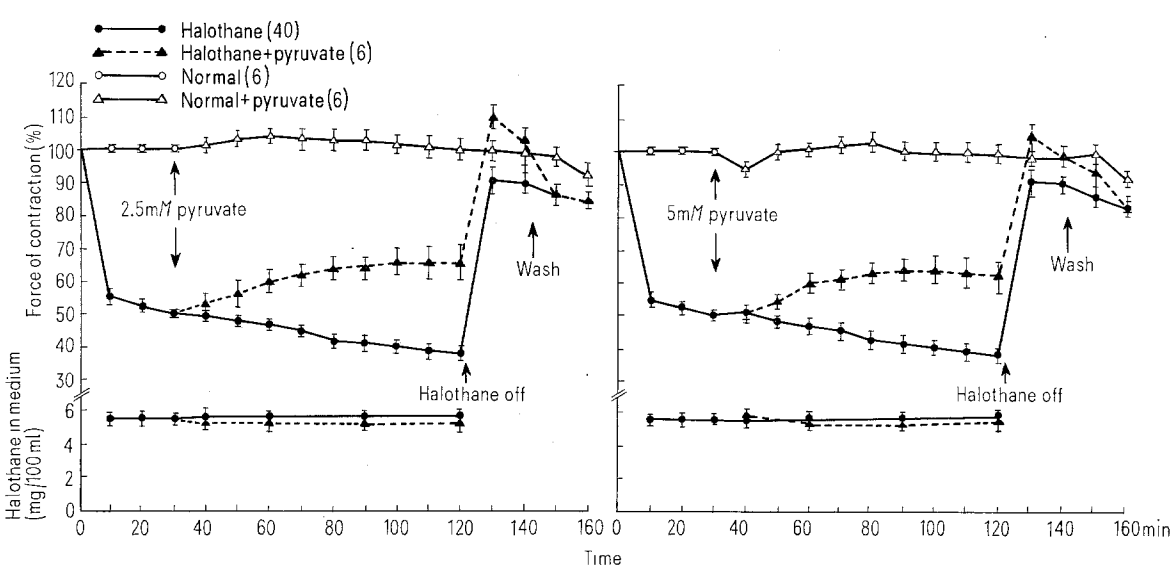

Fig. 1. Effect of 2.5 and 5 m*M* sodium pyruvate on halothane-depressed atria and normal atria. In this and subsequent figures halothane was added at zero time (i.e. following a 60 min equilibration period in the normal Krebs-Ringer bicarbonate glucose medium). At arrow, pyruvate added 30 min after start of halothane administration to halothane depressed atria, and to normal atria. Vertical bars represent the S.E.M.

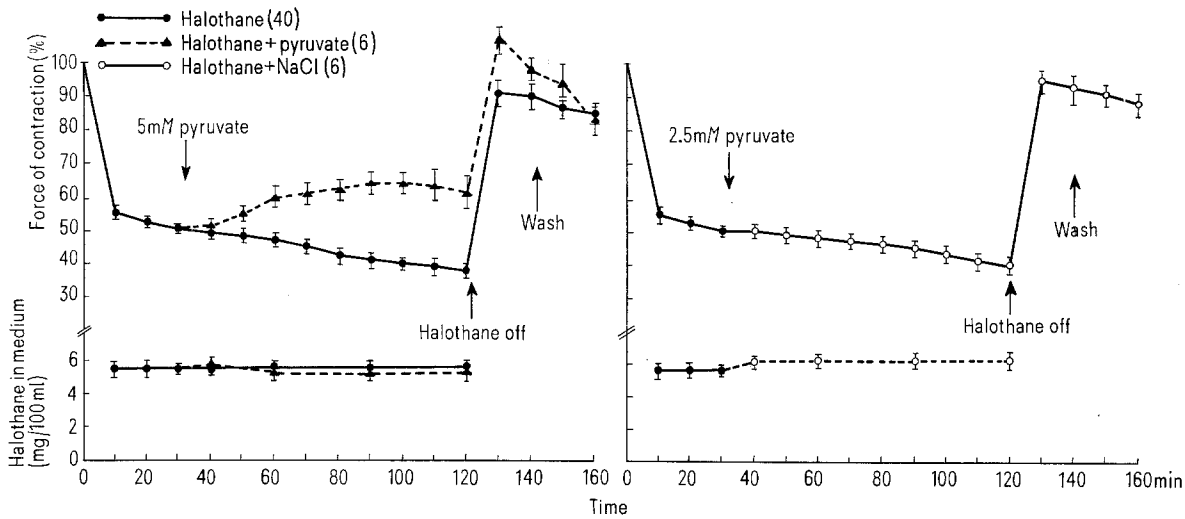

Fig. 2. Effect of 5 mM sodium pyruvate and 5 mM NaCl on halothane-depressed atria.

medium was used rather than halothane to depress the atrial contractility. After 60 min of exposure to this medium, the force of contraction of atria stabilized at approximately 65% of the control levels.

The addition of pyruvate at this time resulted in no increase in contractility (Figure 3). The results indicate that depression of force per se is not a sufficient condition to permit pyruvate to effect a recovery in contractility.

Discussion. Several investigators have been able to demonstrate the relative abilities of various substrates to maintain the contractile activity of myocardial function[13–16]. It is emphasized that efficient operation of energy mechanisms is essential for cardiac function. Thus, cardiac depression and failure must be explained ultimately on a biochemical and/or biophysical basis.

The demonstration in the present study that pyruvate was partially effective in restoring the contractile activity of the isolated rat atria in the hypodinamic state by halothane, has interesting implications of a metabolic role for the action of halothane on cardidac function. It has been reported with rat atria[17] and rabbit atria[18] that either the uptake of glucose or operation of the glycolytic pathway are important for a fraction of contractile activity since pyruvate is partially effective in restoring the developed tension in the absence of glucose or during block with enzyme inhibitor. Similar investigations have been made by Ko and Berman[19], in which they indicate that pyruvate partially restored the depressed contractility of isolated rat atria in phosphate medium either with or without glucose. Pyruvate was relatively effective as an energy source for contraction of isolated rat heart in

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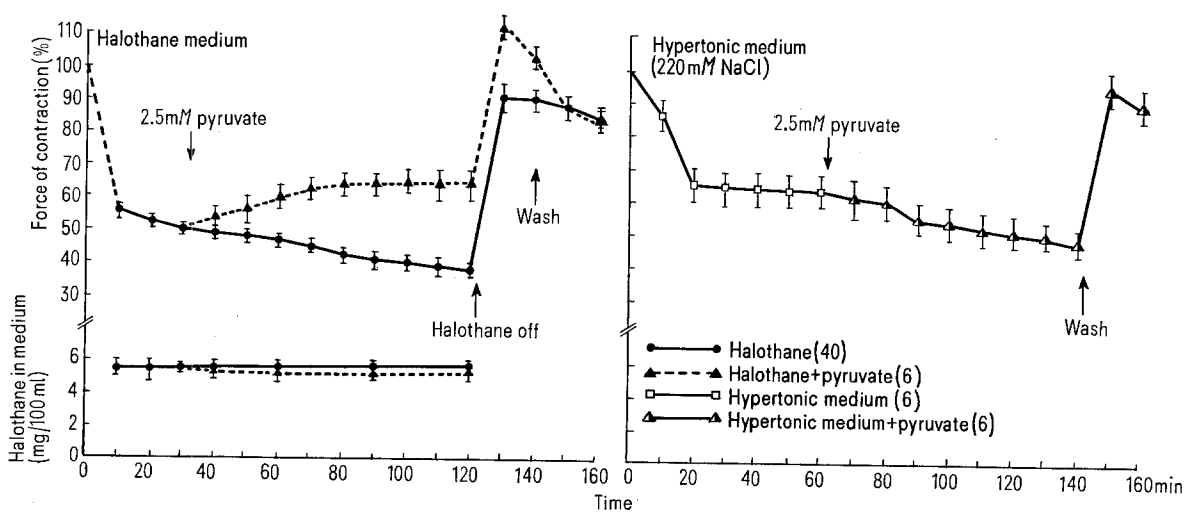

Fig. 3. Effect of 2.5 mM sodium pyruvate on depressed atria with halothane or hypertonic medium.

phosphate medium[20], and in this medium glucose metabolism was impaired in rat heart[21]. SHAW and STADIE[22] proposed that in diaphragm muscle the Embden-Meyerhof pathway was inhibited at the phosphofructokinase step when the tissue was incubated in phosphate medium. If it could be assumed that the effect of pyruvate on halothane-depressed rat atria may be similar to that above, our investigation might be concerned with the manner in which halothane influences the myocardial metabolism. Either the uptake or utilization of glucose may be impaired by halothane. Using partition coefficient data of LARSON et al.[23], a saline concentration of 6 mg/100 ml halothane would be equivalent to a blood concentration of 19.8 mg/100 ml. Blood levels of 17.9–20.3 mg/100 ml were found necessary to anesthetize a dog to produce a loss in the pain reflex of foot pad[12]. Thus, the concentration of halothane employed in this study was similar to that assumed to produce in the dog.

Zusammenfassung. Die nach Halothan eingetretene Verminderung der Kontraktilität des Myokards kann durch Pyruvat verhindert werden. Dieses Ergebnis stützt die Hypothese, Halothan behindere die Aufnahme oder die Verwertung von Glycose.

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Some New Aspects in Human Trophoblast Cultures

Studies concerning the immunological aspects of pregnancy strongly depend on maintaining trophoblast cells in vitro[1–6]. In the present work, a simple method is given for keeping human trophoblast cultures active for a longer period of time, and the morphological evolution of these cells, as well as their hormone secreting activity, during in vitro culture, is described.

The cultures were performed from sterile dissected chorion villi, washed in sterile 199M[7], and carefully cut with fine scissors and forceps. The fragments were transferred to a sterile tube with a rubber stopper, and 0.25% trypsine was added. The tubes were left for about 30 min in incubator at 37°C, shaken for 1 min every 5 min, and then centrifuged. The sediment, washed 2–3 times with phosphate buffer saline until supernatant remained clear, was resuspended with 199M containing 10% sterile calf serum, filtered through sterile gauze, and suspension adjusted to a proportion of 3×10^5 cells/ml[3]; an amount of 1 cm[3] was put into each sterile tube, and left at 37°C. Two types of culture tubes were used: regular ones and Leighton tubes[8], which allow the cells to grow on a removable glass cover slip. Parallel cultures were performed from same cell suspension, in sterile Petri dishes incubated in a CO^2 incubator. The tubes and the Petri dishes were examined every day in the inverted microscope. Cover slips from the Leighton tubes were removed at different intervals. Some of the cover slips were examined without staining in the phase contrast microscope, the others were stained by the May-Grunwald-Giemsa technique, and examined in the bright field microscope. Samples of growth media (199M/10% sera) were taken after 10 days of incubation and kept at 4°C. On about the 20th day, the growth media was removed and replaced by maintenance media (199M without sera). The maintenance media was changed at different intervals. All the removed media samples were concentrated by alcohol-ether extraction, and presence of humanchoriongonadotropin tested by the aid of the haemagglutination inhibition test (HIT)[9].

The development of the Petri dish cultures in CO^2 atmosphere was rapid but limited, and degenerated cells could be observed 2 to 3 weeks after incubation. In contrast to this, the cultures performed in tubes could be kept alive for 2 months and more. These cultures, performed in regular tubes as well as in Leighton, developed rather rapidly. Several aspects could be noted at different intervals.

During the 24–48 h of incubation, multinucleated giant cells and many epitheloid polygonal cells were observed, as well as few fibroblast cells.

The multinucleated giant cells contained about 10–30 nuclei in a pale stained protoplasma, and could be found until about the 8th day of culture (Figures 1–4). Serial examination of cover slip cultures, in the 2nd week, showed groups of cells congregating closely and similar to those included in the 'multinucleated giant cells', but without being delimited by a common external membrane. Their protoplasma was small as compared to the nucleus and increased later. These cells seemed to be remainders of the prior multinucleated giant cells. (Figures 5 and 6).

The epitheloid polygonal cell had a relatively large nucleus with dense and strongly stained chromatine and sometimes with big nucleolus (Figures 7–9). These cells change their shape during the in vitro evolution. At 6–9 days, the protoplasmic edges were more elongated and sometimes curved (Figures 10 and 11). After the 2nd week of culture, these cells were larger, with large nuclei full of

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