pH Dependent Changes in Cell Membrane Stability (35251)

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Resistance to standard homogenization of liver and kidney cells of dogs in hemorrhagic shock has been observed in our laboratory (1). Whereas homogenization of normal liver and kidney in isotonic sucrose or Tris buffered saline (pH 7.5) solutions resulted in complete cell disruption, similar processing of liver and kidney from animals in hemorrhagic shock yielded 35–95% unbroken cells. This effect was subsequently reproduced in nonhypotensive dogs in respiratory acidosis (arterial pH 6.9). The present study was designed to study the effect of an acid medium *in vitro* on normal liver cells.

Methods. Three healthy adult mongrel dogs were killed by rapid exsanguination. The liver biopsy samples were placed in cold (4°) isotonic sucrose solution containing 1 m*M* EDTA and buffered to pH 7.4 with Tris HCl (5 m*M*). All subsequent oprations were performed at 4°.

All studies were done in duplicate on 2-g aliquots of liver parenchyma which was minced, freed of connective tissue, and washed free of blood with the pH 7.4 Tris–sucrose–EDTA solution.

Control minces were incubated for 15 min in 20 ml of this sucrose solution. Three experimental preparations for each dog were incubated for 15 min in 20 ml of Tris–sucrose–EDTA solution buffered to pH 6.9. One set of duplicate minces was homogenized immediately following this treatment. After the initial incubation, the second set was transferred to a similar volume of pH 7.4 solution and reincubated for 15 min. A final set was reincubated in Tris–sucrose–EDTA solution buffered to pH 8.2. Following reincubation, these sets were homogenized. The pH was determined at the conclusion of each incubation and reincubation to exclude changes secondary to leakage of intracellular contents. In preliminary experiments, 15-min incubations and reincubations were repeated at 30 and 60 min and acid exposed minces were incubated at pH 6.2 as well as 6.9. Subsequently, all incubations were for 15 min and a pH 6.9 acid environment was used exclusively. The experimental protocol is shown schematically in Fig. 1.

Tissues were homogenized in a Dounce homogenizer (Blassig Glass Co., Rochester, N.Y.) using 10 strokes each of the loose and tight pestles. After homogenization, samples were filtered through 2 layers of cotton gauze. Filtrates were examined for unbroken cells using phase contrast microscopy at 400× magnification. The total number of nuclei and unbroken cells in 10 microscopic fields were counted. The percentage of intact cells was determined by the ratio:cells ÷ cells + nuclei.

Results. Initial data following incubation at 15, 30, 60 min and at pH 6.2 and 6.9 showed no significant differences. Therefore, results will be reported for the 15-min incubations at pH 6.9 only.

Table I shows that incubation at acid pH renders almost half the cell population resistant to homogenization. Furthermore, this

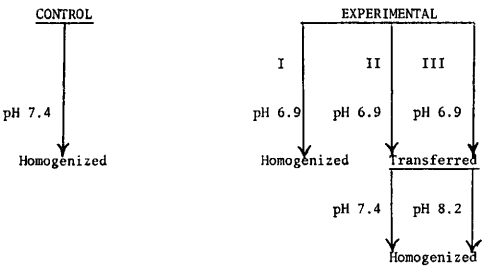

FIG. 1. Schematic representation of experimental protocol.

TABLE I. Comparison of the Percentage of Unbroken Cells Present in Homogenates After Incubation of Liver Minces at pH 7.4 (control), at pH 6.9, and Reincubation at Higher pH (7.4 and 8.2) of Samples Initially Exposed to an Acid pH (6.9).[a]

		Experimental		
	Control	No reincubation	Reincubation	
Dog no.	pH 7.4	pH 6.9	pH 7.4	pH 8.2
1	0, 0	45, 40	25, 21	3, 4
2	7, 2	45, 30	29, —	6, 14
3	7, 6	37, 39	11, 12	7, 9
Mean	3.7	39.4	16.3	7.2

[a] Values for the duplicate determinations are listed.

change appears to be partially reversible at physiologic pH (7.4) and nearly completely reversible at pH 8.2. Microscopic examination of homogenates following incubation at pH 6.9 showed effective disaggregation of cells without disruption, an appearance similar to that occurring *in vivo* with shock or respiratory acidosis.

Discussion. This study demonstrates an apparent increase in plasma membrane stability after exposure of normal liver cells to an acid environment. This effect was similar in microscopic appearance and magnitude to that we observed with shock (hypotensive hypovolemia) and acidosis *in vivo*. The pH at which it occurred was the same as the arterial pH of the intact dogs previously studied. The effect was reversible on reincubation of resistant cells at higher pH. Reversibility increased with increasing pH of the reincubation medium from pH 7.4 to 8.2. These findings lend added credence to our previous contention that acidosis is responsible for the occurrence of this phenomenon in shock (1).

While membrane chemists have stressed the necessity of controlling pH in preparing homogeneous and reproducible plasma membrane fractions (2), little is known about the mechanism of pH dependent changes in membrane structure. Curtis demonstrated greater adhesion of corneal epithelium to the connective tissue matrix at acid pH (3). These data seem unrelated to our findings since our cells were almost completely disaggregated yet remained unbroken. Dodge *et al.* (4) found the size of membrane fragments from erythrocyte ghosts to be inversely related to the pH on homogenization, suggesting a resistance to lysis at lower pH. Even more relevant was the unpublished observation of T. L. Steck and D. F. H. Wallach that in preparation of erythrocyte ghosts, cytoplasm adhered to membrane fragments at acid pH. Such adherence may lend abnormal physical stability to the membrane.

The resistance of acidotic cells to homogenization has great practical significance in the interpretation of biochemical data derived from tissue homogenates of animals in shock. Isolation and study of subcellular components from incompletely homogenized tissues can lead to conclusions based on a selected population of more fragile cells. For example, conflicting reports have appeared on the stability of rat liver lysosomes in shock (5, 6). Since a buffered sucrose medium was used in one study (5) but not in the other (6) the results were not comparable. Use of uniformly buffered homogenization media and inspection of homogenates by phase contrast microscopy may avoid such pitfalls.

Summary. A pH dependent, reversible resistance of normal dog liver cells to standard homogenization has been demonstrated *in vitro*. This phenomenon is very similar to that previously observed in dogs in hypotensive hypovolemia or respiratory acidosis. Possible mechanisms by which acid pH may alter plasma membrane stability have been discussed. The significance of this phenomenon in interpretation of biochemical data derived from homogenates of shocked tissues is emphasized.

1. Lazarus, H. M., Herman, A. H., Bell, M. L. Rutenburg, A. M., and Egdahl, R. H., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 2638 (1970).

2. Dowben, R. M., *in* "Biological Membranes" (R. M. Dowben, ed.), p. 2. Little Brown, Boston (1969).

3. Curtis, A. S. G., Biol. Rev. **37**, 82 (1962).

4. Dodge, J. T., Mitchell, C., and Honahan, D., Arch. Biochem. **100**, 119 (1962).

5. Janoff, A., Weissman, G., Zweifach, B. W., and Thomas, L., J. Exp. Med. **116**, 451 (1962).

6. Schumer, W., Kapica, S. K., and Teng, T. L., Arch. Surg. **99**, 325 (1969).

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