Cellular Stability in Experimental Hemorrhagic Shock[1] (35709)

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In the course of a study of subcellular components in hemorrhagic shock in the dog, we observed that liver and kidney cells showed resistance to homogenization. The present report deals with this increased cellular stability in experimental shock.

Materials and Methods. Hemorrhagic shock in 12 dogs was induced by the reservoir technique (1, 2). Liver and kidney biopsies obtained before, during, and after the hypotensive period were immediately placed in a freezer at —20°. Three to six months later the frozen tissues were processed. Fresh biopsy specimens were also examined from three additional dogs in shock. One-gram aliquots were minced and homogenized (10 strokes) in 10 vol of 0.01 *M* Tris–HCl (pH 7.5)–0.01 *M* NaCl solution, in a standard loose Dounce homogenizer (Blaessig Glass Co., Rochester, N.Y.).

This crude homogenate was then filtered through a monofilament nylon 103 mesh (approximately 200-μ openings) to eliminate fibrous tissue. The filtrate, containing cells and cellular contents, was rehomogenized (10 strokes) in a standard tight Dounce homogenizer. In addition, other methods of homogenization were utilized, including: ultrasonic treatment (3), freezing and thawing, homogenization in distilled water and 1% Triton N 101 (Rhom and Haas, Wellesley, Mass.), and nitrogen cavitation. (4)

For nitrogen cavitation, the crude homogenate of cells was equilibrated with nitrogen at 800 psi in a modified hydrogenation bomb for 20 min and rapidly restored to atmospheric pressure by ejection through a large-bore nozzle. Once atmospheric pressure is restored, small nitrogen gas bubbles form near the cell surface, producing localized shearing force sufficiently powerful to lyse cells.

Six additional dog experiments were designed to assess the effect of acidosis, alkalosis, and hypercortisolemia on cellular stability. Thus, liver specimens were obtained and processed as above from two dogs in hemorrhagic shock and acidosis (pH 7.1) both before and after induction of alkalosis (pH 7.8) with intravenous Tris buffer; from two normotensive dogs in respiratory acidosis (pH 6.9) induced by rebreathing of carbon dioxide; and from a dog given hydrocortisone (1 g) intravenously and a dog given ACTH (20 units) intramuscularly.

Wet smears of the homogenate were examined by phase-contrast microscopy for completeness of cell disruption at × 100 and 400 routinely and × 1000 for photomicrographs. The result was expressed as a ratio of the number of intact cells to the total cells plus nuclei.

Results. The homogenates of normal liver and kidney, stored at —20° for 3–6 months, consisted almost entirely of free nuclei and cytoplasmic contents. Whole cells were rarely seen. In all preshock specimens less than 10% of the liver cells and less than 1% of the kidney cells remained intact after homogenization. Sequential tissue biopsies revealed that after hypotension for 30 min, at a mean arterial blood pressure (MABP) of 35 mm of mercury, whole undisrupted but disaggregated cells were present as well as free nuclei. After 1 hr of hypotension even more cells became resistant to disruption (Figs. 1 and 2), reaching a plateau which was maintained throughout several additional hours of hypo-

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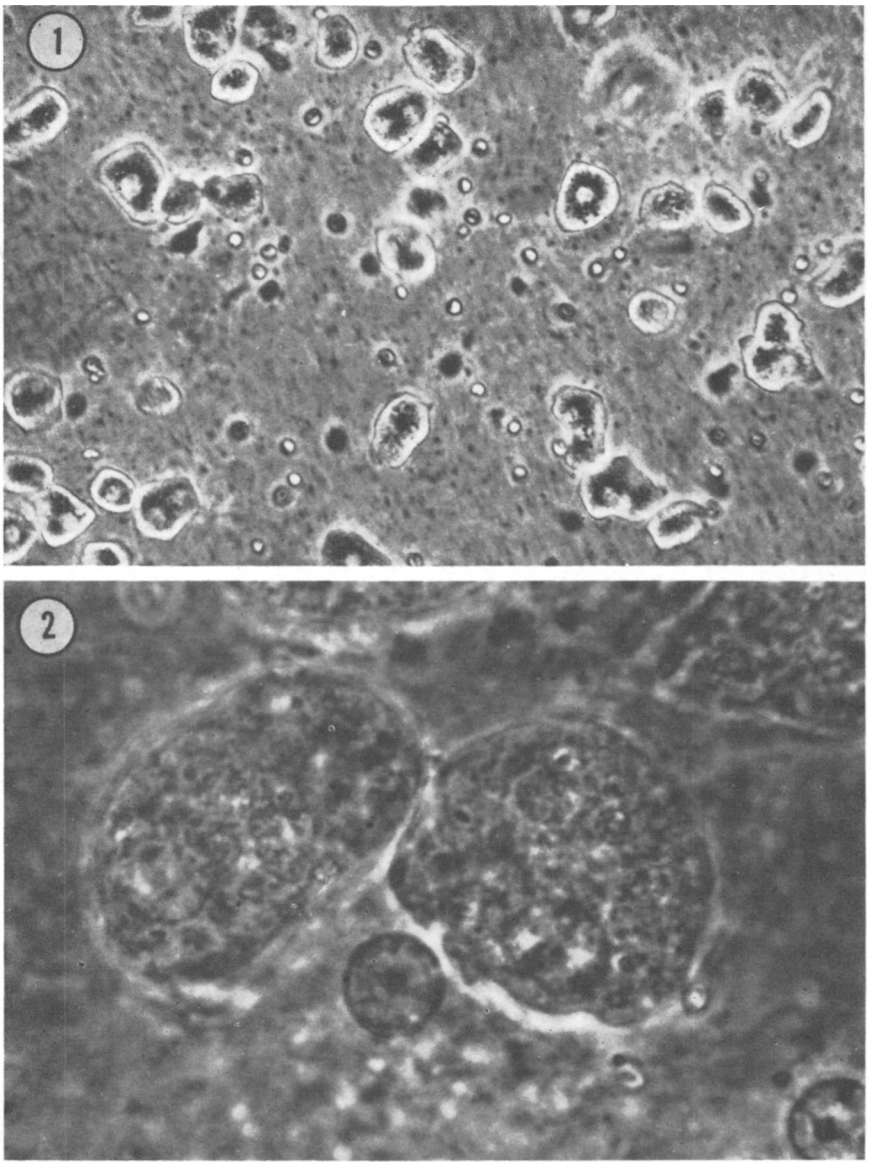

FIG. 1 and 2. Homogenate of dog liver 1 hr past reinfusion after the animal had taken back 35% of his shed blood volume. Figure 1, ×200, many whole cells are present along with the nuclei and cytoplasmic granules. Figure 2, ×1000, the intracellular nuclei of the intact cells and the free nuclei are readily appreciated at this magnification.

tension. The kidney homogenate showed fewer resistant cells and more free nuclei at × 1000 magnification. In all but 1 of the 12 dogs subjected to hypovolemic hypotension, 76–98% of liver cells and up to 22% of renal cells were resistant to homogenization (Table I).

Ultrasonic treatment, freezing, and thawing, homogenization in distilled water, and homogenization in 1% Triton N101 were also ineffective in disruption of the resistant cells from shocked animals. Only the nitrogen cavitation technique disrupted more than 90% of liver or renal cells biopsied from shocked dogs resistant to standard homogenization. In two normovolemic normotensive

TABLE I. Cell Membrane Resistance After Hemorrhagic Shock.

Degree of hypotension[a]	Hours of hypotension	Biopsy time	Percentage of intact liver cells
Mild	1.2	4 hr after shock	79
Mild	1.3	4 hr after shock	82
Mild	18.3	Terminal	91
Mild	34.6	Terminal	98
Moderate	8.2	Terminal	84
Moderate	10.0	Terminal	90
Moderate	16.1	Terminal	82
Moderate	20.6	Terminal	93
Severe	1.3	Terminal	96
Severe	1.5	Terminal	76
Severe	2.0	Terminal	98
Severe	8.3	Terminal	1

[a] Mild refers to hypotension with MABP $<$ 100 mm Hg but $>$ 65 mm Hg; or to an MABP $<$ 65 mm Hg but $>$ 50 mm Hg, if time $<$ 4 hr. Moderate refers to hypotension with MABP $<$ 65 but $>$ 50, if time $>$ 4 hr. Severe refers to hypotension with MABP $<$ 40 mm Hg.

dogs, laparotomy alone or when followed by intravenous ACTH (20 units) or hydrocortisone (1 g) did not increase the resistance of liver cells to homogenization.

Based on an evaluation of cell disruption and membrane isolation techniques (5), Steck suggested that the lowered cellular pH occurring in shock might itself be responsible for the resistance to homogenization. Thus, respiratory acidosis (arterial pH 6.9) induced in dogs by rebreathing carbon dioxide resulted in increased resistance to homogenization. Similarly, in *in vitro* experiments we observed that exposing liver cells to pH 6.9 induced increased resistance which could be reversed by reincubation at pH 8.2 (6). In liver freshly removed from dogs in shock or acidosis, the resistance to lysis could be similarly reversed, but not after the storage of the liver tissue at —20° for 3–6 months. When the acidosis (pH 7.1) observed in shock was converted to an alkalosis (pH 7.8) by intravenous infusion of Tris buffer the prior resistance of hepatocytes to lysis was reversed.

Discussion. Data herein presented indicate that liver and kidney biopsies from dogs in hemorrhagic shock showed increased resistance to cellular disruption by standard techniques of homogenization. Cancer was another pathological state which produced cells resistant to homogenization in standard sucrose media (7). These tumor cells could be lysed with detergent solutions (8). However, this was not the case with liver cells biopsied from dogs in hemorrhagic shock. The alteration of the physical characteristics of the cells described herein appeared to be promoted by acidosis. Cellular as well as extracellular acidosis are generally encountered in shock (9–12). We conjecture that the observed cell changes are caused by a "toughening" of the plasma membrane induced by the acidosis (arterial pH of 6.9–7.1) in shock. Whether this was due to an irreversible adherence of cytoplasm to the inner surface of this membrane, as suggested by experimental observations of Steck (5), or to an actual structural membrane change, could not be established within the scope of this report.

If a method of homogenization leaves a significant portion of cells unbroken, the subcellular particles freed in the homogenate would reflect only a select segment of the cellular population rather than the entire organ, a segment that is, perhaps, least affected by the pathologic state under study. Thus, the failure of standard homogenization to release all intracellular components after shock may pose a problem in the interpretation of biochemical changes in subcellular particles. The phase-contrast microscope makes possible rapid assessment of the percentage of cells being lysed.

Summary. Hemorrhagic shock of varying severity caused liver and kidney cells to become resistant to standard homogenization. This change, readily detected by phase-contrast microscopy, was in part due to the metabolic acidosis of shock.

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