BSS Plus: a potential irrigating solution for neurosurgery

GLENN A. MEYER, M.D., DENNIS J. MAIMAN, M.D., HENRY F. EDELHAUSER, PH.D., O. J. LORENZETTI, PH.D., AND JOHN GARANCIS, M.D.

Departments of Neurosurgery, Physiology, and Pathology, Medical College of Wisconsin, and Veterans Administration Medical Center, Milwaukee, Wisconsin

BSS Plus is a pH-stable balanced salt solution similar to glutathione bicarbonate Ringer's solution. Extensively used in ophthalmology, it is of potential value in neurosurgery. In comparative tests of its effectiveness, 28 cats underwent bilateral irrigation of the surface of the cerebral cortex with normal saline on one side and BSS Plus on the other. After 2 hours, a marked decrease was seen in the surface pH of the hemisphere irrigated with normal saline but not of the hemisphere treated with BSS Plus. Blood-brain barrier changes (measured with Evans blue dye techniques) were more evident following saline irrigation. Somatosensory evoked potentials and cerebral blood flow were not significantly altered. Conventional light microscopy using three standard stains did not reveal a significant difference. Transmission electron microscopy studies were performed in 14 animals and scanning electron microscopy in six. In five animals both transmission and scanning electron microscopy studies were conducted after irrigation with both agents without a cottonoid cover and with immediate harvest of superficial layers from the living brain and immersion-fixation in glutaraldehyde. Tissue preservation was superior on the BSS Plus side in all studies. This agent may represent an improved irrigation solution for neurosurgery, but further studies are required.

KEY WORDS: irrigation solution, BSS Plus, blood-brain barrier, electron microscopy, cat

NORMAL saline is the solution traditionally used in neurosurgery for irrigating wounds, in part because of its low cost and ready availability. However, the use of saline has several theoretical disadvantages which may be of unrecognized clinical importance. The pH of saline ranges from 5 to 7 and is unbuffered; therefore, it can cause an acid environment. In the absence of circulating cerebrospinal fluid, as in most open neurosurgical procedures, this acid pH likely contributes to adverse tissue changes in the surface layers of the exposed brain and spinal cord. 12,17

Experimental studies by Edelhauser, et al., 5,6 on saline perfusion of corneal endothelium have shown that this agent contributes to cell death. Marked increase in total corneal thickness was evident. Breakdown of intercellular tight junctions and necrotic changes in the endothelial cells occurred after 90 minutes of saline perfusion. Other solutions tested, including lactated Ringer's solution and Plasma-lyte,* have shown similar results. 3,4 These solutions are all unbuffered. Lactated Ringer's solution can also have an acid pH, and Plasma-lyte lacks calcium; both of these factors create an environment that tends to cause breakdown of intercellular tight junctions. 8 These considerations are potentially significant in the central nervous system (CNS), as abnormalities of the cellular junction may be related to edema 2 and injury to the blood-brain barrier. 16

BSS Plus,† a bicarbonate-buffered and balanced irrigation solution containing calcium, glucose, and glutathione (Table 1), has been demonstrated to have

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* Plasma-lyte is manufactured by Travenol Laboratories, Deerfield, Illinois.
† BSS Plus is manufactured by Alcon Surgical Products, Fort Worth, Texas.
were positioned with a micromanipulator with the aid of a micromanipulator and the cortical surface was examined with an operating microscope for evidence of extravasation indicating injury to the blood-brain barrier. In another two animals, the respirator was turned off for 4 minutes after the minimum 4-hour irrigation period to determine the effect of hypoxia on the parameters measured. The pH and SEP’s were recorded every 15 minutes for the next 3 hours.

Tissue from 12 animals was processed by the conventional method for light microscopy. Alternate sections were stained with Weil, hematoxylin and eosin, and Nissl stains, and 128 slides were evaluated by the investigators and a consultant neuropathologist. Transmission electron microscopy (TEM) was performed in 14 animals. In nine of these, in situ perfusion was performed through neck vessels using initial saline washout of blood followed by glutaraldehyde fixation. In one of these nine animals, scanning electron microscopy (SEM) was also performed. In the five other animals, the irrigation conditions were modified as follows: no attempt was made to remove the arachnoid, and a small strip of cottonoid was placed on the dura just above the area of dural resection. The drip rate was increased to allow a continuous flow of BSS Plus and saline over the exposed arachnoid membrane and underlying pia mater. The area was inspected periodically with the operating microscope to insure that no surface drying occurred. Superficial layers of the leptomeninges and brain were removed from the intact animal with a sharp razor blade. The tissue was quickly washed with saline and dropped into glutaraldehyde for immediate fixation.

Results

Summary of Changes

Changes in surface pH as a function of time are depicted in Fig. 1. Marked depression in pH was seen at 1½ to 2½ hours with saline irrigation. Changes in pH related to saline irrigation became statistically significant at approximately 2 hours (F = 2.9) and had increased markedly at the 4-hour termination point (F = 21.3). On the BSS Plus side, no significant changes in pH were seen. The difference in pH between normal saline and BSS Plus became significant at 2½ hours (F = 4.59) and had more than doubled by termination of the trial (F = 11.2). The difference was even more apparent when values were plotted as change from baseline pH over time (Fig. 2).

In the two animals with induced hypoxia, decreases in pH were seen on both sides but were more marked on the saline-irrigated surface. These changes resolved over the 3-hour measurement period on the BSS Plus-treated surface; on the saline side, the pH continued to decrease. Gross brain swelling occurred bilaterally.

### Materials and Methods

A total of 28 mongrel cats were studied in three series of experiments. The animals were anesthetized with Surital (sodium thiamylal) and atropine, and were maintained on animal ventilators. Intravenous and intra-arterial catheters were placed via a femoral artery cut-down procedure, and blood pressure, temperature, and arterial blood gases were monitored. Bilateral craniectomies were performed, and the dura was elevated from the cortex using microsurgical techniques. Three platinum disc-electrodes were placed in line laterally on the exposed cortex to measure evoked potentials; these were then recorded on an averaging computer. The pH was monitored bilaterally in 17 cats, using pH electrodes and standard pH meters.† The pH electrodes were positioned with a micromanipulator with the aid of an operating microscope. Local cerebral blood flow (CBF) was monitored using the hydrogen clearance method.†

Immediately after exposure of the cortical surface, continuous-drip irrigation by small-bore needles was initiated at a rate of 30 to 50 cc/hr to saturate the cortical surface. At the same drip rate, 0.9% saline was applied on one side and BSS Plus on the other for a minimum of 4 hours in each animal. Each bottle was maintained at room temperature (22°C).

| TABLE 1  
Chemical composition of BSS Plus and normal saline |
<table>
<thead>
<tr>
<th>Factor</th>
<th>Cerebrospinal Fluid*</th>
<th>BSS Plus†</th>
<th>0.9% Normal Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>cations (mEq/liter)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Na⁺</td>
<td>145.0</td>
<td>150.7</td>
<td>154</td>
</tr>
<tr>
<td>K⁺</td>
<td>3.0</td>
<td>5.1</td>
<td>0</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>2.2</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>2.3</td>
<td>2.1</td>
<td>0</td>
</tr>
<tr>
<td>anions (mEq/liter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPO₄⁻</td>
<td>1.0</td>
<td>3.5</td>
<td>0</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>125.0</td>
<td>131.4</td>
<td>154</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>24.0</td>
<td>25.0</td>
<td>0</td>
</tr>
<tr>
<td>pH</td>
<td>7.35 ± 0.1</td>
<td>7.2 ± 0.4</td>
<td>6.0 ± 1.0</td>
</tr>
<tr>
<td>osmolality (mOsm)</td>
<td>306 ± 10</td>
<td>305 ± 15</td>
<td>308</td>
</tr>
</tbody>
</table>

† BSS Plus also contains two non-ionic components: 5.1 mMol dextrose and 0.28 mMol oxidized glutathione.
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along with the post-hypoxia pH changes. These were partially reversed during normal ventilation (coinciding with the pH changes) on the BSS Plus surface, but remained unchanged on the saline side.

Cerebral blood flow was measured in five of the initial six cats. There were minor changes (not statistically significant) in CBF on the saline sides associated with marked changes in pH. Similarly, SEP changes were not pronounced.

Blood-brain barrier changes were prominent in three of the four animals injected with Evans blue dye. There was no dye extravasation on the BSS Plus-irrigated surfaces, except in one animal with a small pial tear. Changes were marked on the saline-treated sides, with multiple areas of breakdown in two animals and confluent dye extravasation in the third.

The histological review of the analysis of the 128 slides was disappointing. Our studies with three conventional stains did not show any consistent differences in tissue damage. The amount of tissue disruption in different areas of the exposed cortex was highly variable, very likely due to the degree of adherence of the cotonoid sponge which remained in contact with the pia arachnoid surface during the 4-hour period of irrigation. While the studies were suggestive of better preservation of tissue on the BSS Plus side, scoring on a 0 to 4+ system did not reveal any significant difference in the two sides.

In nine of the animals studied histologically, TEM was also performed on the surface layers. The results were again unrevealing. A 1 to 4+ scoring system was designed to assess changes in the following tissues: the synapse, synaptic vessels, axons, myelin sheath, and areas of focal necrosis. In six of these animals, tissue processing was sufficiently uniform to allow accurate comparison of the two sides. Again no significant difference was found. A pilot study in one of the animals using SEM techniques clearly revealed a better result on the BSS Plus side. In the five animals with intact pia arachnoid, tissue preservation was uniformly better on the BSS Plus side following the 4-hour irrigation interval.

One animal was utilized for control purposes. Tissue samples were harvested immediately after exposure of the brain surface and processed routinely. The resulting photomicrographs were of excellent quality and showed only normal microanatomy.

Electron Microscopy

Saline-Irrigated Hemisphere. On TEM, the surface lining cells of the arachnoid showed various degrees of necrosis and stained less intensely than the better preserved deeper cells (Fig. 3 left). The most superficial cells had extensive disruption of cytoplasm; only a few mitochondria and cytoplasmic fragments remained attached to the nucleus. Nuclear changes consisted of coarse chromatin clumping. The less affected, deeper cells showed diffuse dilatation of endoplasmic reticulum. Vascular changes of the arachnoid were limited to the endothelial and smooth-muscle cells. Widening of the intercellular junctions of the endothelial layer was present in capillaries and arterioles. There was vacuolization of the endothelial cells, most prominent in the arterioles and capillaries (Fig. 4 left). Pinocytotic vesicles were observed in the smooth-muscle cells of the arterioles (Fig. 4 left). Evidence of interstitial edema was seen in the wide dispersion of collagen fibers.

Ultrastructural changes were observed in the astrocytes, superficial oligodendrocytes, and axons on the cerebral surface. In general, less affected cells showed mild to moderate intracytoplasmic edema characterized by diffuse dilatation of endoplasmic reticulum. In some areas, the damage to astrocytes and oligodendrocytes was more severe. In addition to dilatation of endoplasmic reticulum, there was focal cytoplasmic necrosis. Nonmyelinated axons showed cystic degeneration, accompanied by loss of mitochondria, microtubules, and neurofilaments (Fig. 5 left).

The undulating appearance of the arachnoid surface representing linear arrangement of the surface cells was
FIG. 3. Transmission electron micrographs of the arachnoid.  
Left: Saline-treated sample showing the upper layer of surface lining cells (C) partially necrotic. Those cells are stained less intensely than are better preserved deeper cells (D). All superficial cells show disruption of the cell membrane (arrows) and in some cells extensive cytoplasmic necrosis is present (X). F = collagen fibers, L = capillary lumen. × 4800.  
Right: Sample irrigated with BSS Plus solution. The surface lining cells (C) are intact and form long intertwining cytoplasmic processes (arrow). Only a few small cytoplasmic vacuoles (V) are present. × 6950.

lost in the saline-irrigated specimens on SEM. Delineation of individual cells was widely effaced; only disrupted cytoplasmic bands were recognized (Fig. 6 left).

**BSS Plus-Irrigated Hemisphere.** The surface lining cells of the arachnoid were intact on TEM. The only noticeable change in these cells was slight separation of the cytoplasmic processes and the presence of small cytoplasmic vacuoles (Fig. 3 right). The endothelial cells of the capillaries and arterioles appeared essentially normal (Fig. 4 right). There was no apparent interstitial edema. Clusters of collagen fibers were more densely distributed than in the saline-treated specimens. The cerebral surface astrocytes and the underlying oligodendrocytes showed normal ultrastructure. Myelinated and nonmyelinated axons appeared essentially normal (Fig. 5 right).

Scanning electron microscopy showed that the surface of the arachnoid had a fine undulating appearance due to parallel arrangement of the surface lining cells. It appeared that the loosened cytoplasmic processes formed free-floating membrane-like structures (Fig. 6 right).

**Physical Compatability Studies**

In preparation for clinical trials, physical compatibility studies were performed by one of the authors.

FIG. 4. Transmission electron micrographs showing arachnoid arterioles.  
Left: In this saline-irrigated specimen, the endothelial cells contain large cytoplasmic vacuoles (V). Increased pinocytosis (arrow) of pericytes is present. E = erythrocyte. × 13,500.  
Right: BSS Plus-treated specimen. The cytoplasm of the endothelial cells (E) contains a few pinocytotic vesicles (arrowhead). The intercellular junction (arrow) appears intact. L = lumen of arteriole. × 15,500.
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FIG. 5. Transmission electron micrographs of the cerebral surface. Left: In the saline-treated specimen, astrocytes (C) and superficial oligodendrocytes (O) exhibit focal cytoplasmic necrosis (N). There is cystic degeneration of nonmyelinated axons (A). M = myelinated axon. × 6265. Right: BSS Plus-treated specimen showing surface astrocyte (A) with intact cytoplasmic processes (P). Underlying oligodendrocytes contain myelinated axons (M) and few nonmyelinated axons (N). All axons appear intact. × 6175.

(O.J.L.) at the Alcon Laboratories. Various solutions were prepared according to the manufacturers' recommendations, combined with BSS Plus, and subjected to study. No adverse precipitation or pH change was observed in mixing equal parts of BSS Plus with epinephrine, heparin, papaverine, lidocaine, tetracycline, bacitracin, or kanamycin. Although chlorpromazine irrigation is not used in neurosurgery, it is used as an irrigation fluid in vascular surgery, so this agent was also evaluated. A cloudy precipitate resulted and therefore chlorpromazine irrigation fluids will be avoided in clinical studies.

Discussion

We found it instructive to compare our data with the pioneering work of Elliott and Jasper7 reported in 1949 and the findings of Lewis and Elliott11 published the following year. The deficiencies of normal saline as an irrigation fluid, particularly as a fluid to restore the volume of depleted cerebrospinal fluid, are well described. We also noted that the marked variability of subpial pH directly correlated with distance from the pial arterioles. Our results showed that prolonged saline irrigation will cause a decrease in subpial pH and that BSS Plus will maintain pH levels over time despite a wide range of initial pH levels of 7.5 to 7.1 (Figs. 1 and 2). When averaged, these pH data were consistent. Our results agree with the work of Hansson and Vällfors9 with cell and tissue cultures and also with their more clinically oriented studies.18,19 We are in agreement that more physiological irrigation solutions are effective in maintaining the blood-brain barrier and normal appearance of surface layers upon electron microscopy. We did not evaluate the less complex solutions de-

FIG. 6. Scanning electron micrographs of the arachnoid. Left: Saline-treated specimen. The smooth undulating appearance of arachnoid is lost because the surface lining cells (C) are disrupted or damaged. × 165. Right: BSS Plus-treated specimen. The arachnoid has a fine undulating appearance with parallel alignment of the surface lining cells. × 150.
scribed in their reports; namely, Ringer's solution and Elliott's B solution. We do not think that either of these solutions offers all of the advantages of the BSS Plus solution. Although preliminary, our findings tend to substantiate the hypothesis that BSS Plus may represent an attractive irrigation solution for neurosurgical procedures, primarily because it is buffered with HCO₃⁻ (the same buffer as in cerebrospinal fluid) and because it contains glucose and glutathione. The pH changes observed with normal saline were dramatic; although the extent and reversibility of injury caused to the CNS is difficult to assess, some adverse tissue change can be strongly suspected. In addition to dysfunction of enzyme activity and alteration of electrical potentials causing abnormalities of conduction, it is conceivable that secondary ionic shifts might occur at the neuronal level. Swiontek, et al., ¹⁷ have suggested that severe pH changes often represent secondary manifestations of nervous tissue injury.

The changes in the blood-brain barrier were also prominent. It has been observed that the blood-brain barrier is maintained by the endothelial tight junctions, which are protected by the ionic composition and buffer of the plasma. ¹⁵ Several investigators have emphasized that the production of cerebral edema is related to the breakdown of the blood-brain barrier, with gaps occurring at the endothelial tight junctions causing an increase in permeability. ¹⁰ The preservation of the blood-brain barrier in BSS Plus-treated specimens was probably related to maintenance of the endothelial tight junctions regulated by the calcium and bicarbonate buffer present in that agent.

The relatively unchanged CBF values and SEP's that we recorded confirmed our expectations. Probably no changes were seen because the depth of cell injury was insufficient to produce them.

Of particular interest were the two animals subjected to short periods of hypoxia. In both instances, marked pH decreases were seen immediately following the episode. These persisted and became worse on the surfaces irrigated with normal saline; however, on the BSS Plus side, the pH recovered almost to baseline in both animals. In addition, the edema seen shortly after the hypoxic episode was largely reversed in the BSS Plus-irrigated cortex. While it is impossible to establish a mechanism on the basis of these two trials, perhaps reestablishment of the endothelial tight junctions assisted in the resolution of the edema. In one of these two trials, the saline likely contributed to the blood-brain barrier alterations and also to the disruption of cells.

It is evident from the present morphological studies that the BSS Plus solution preserved tissues better than did saline as a wound-irrigating solution. Saline solution produced necrosis of the surface lining cells of the arachnoid (Fig. 3 left) and focal degenerative changes of astrocytes, oligodendrocytes, and nonmyelinated axons in the superficial layer of the brain (Fig. 5 left). Irrigation of the arachnoid with saline solution also induced vascular changes characterized by widening of the intercellular junctions and cytoplasmic vacuolization of the endothelial cells, and by increased pinocytic activity of the smooth-muscle cells (Fig. 4 left). These changes are expected to cause increased vascular permeability.

We believe that our work and that of others establishes that a balanced buffered salt solution is superior to saline as a wound-irrigating solution in maintaining tissue integrity and homeostasis. The question remains as to whether or not this is clinically important. We believe this is a significant question and that further clinical research is warranted.

Conclusions

1. A balanced buffered salt solution was superior to saline in maintaining subpial pH in the physiological range during a 4-hour exposure and bathing of leptomeningal surfaces in the cat brain.

2. Ultrastructural studies demonstrated that a balanced buffered salt solution caused significantly less edema in the subpial tissue than did saline.

3. Further studies including clinical trials are justified since our investigations suggest that BSS Plus may represent an improved irrigating solution for neurosurgical procedures on both a theoretical and experimental basis.

References


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Address for Dr. Lorenzetti: Alcon Laboratories, Fort Worth, Texas.
Address reprint requests to: Glenn A. Meyer, M.D., Department of Neurosurgery, Medical College of Wisconsin, 8700 West Wisconsin Avenue, Milwaukee, Wisconsin 53226.