Impairment of Cell Membrane Transport During Shock and after Treatment

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THE DEVELOPMENT of technics to measure the functions of cells and their organelles has provided the opportunity to determine alterations in cell function which may occur with shock and recover with treatment. Haldane once said that anoxia not only stops the machine, but wrecks the machinery. Circulatory failure is more complex than anoxia or hypoxia alone, but it does eventually wreck the machinery. A number of investigators have dedicated themselves to the task of studying how cell functions are altered by shock. Such studies have included the morphologic changes in cells seen by electron microscopy,[7,14] mitochondrial function,[1,6,17,21,23] lysosomal enzymes,[12] the cyclic-AMP system and nucleic acid synthesis.[15] We have found that with prolonged hemorrhagic shock, mitochondrial metabolic capability is decreased,[1,21] mitochondrial cation content is altered with decreased potassium and increased sodium;[2] the Na-K ATPase activity is increased in the liver[30] and the energy containing compound ATP is greatly decreased in hepatic, renal and skeletal muscle cells.[4] Most of these alterations are reversible by treatment with volume restoration. These changes and the work of others[10,11] suggest that the ability of cell membranes to carry out active cation transport may be impaired during shock.

Maintenance of cationic gradients between cells and their surroundings is vital for the functioning of nerve and muscle cells, for the absorptive and secretory activity of cells of the gastrointestinal tract, and the renal tubules. Normal cationic distribution across cell membranes is also in all probability essential to the maintenance of normal cell volume in most of the cells of the body. Thus, the integrity of cell membrane electrolyte transport must be an important factor in the survival and function of cells.

Shires and colleagues have studied cell membrane function *in vivo* by measurement of transmembrane potential and have found decreases with shock that may persist after treatment.[3, 5, 24, 26] This suggests an alteration in the ability of such cells to maintain normal ionic gradients. At the same time, we have studied cell membrane transport in an *in vitro* system to try to measure the capability of the transport system more directly, and have reached similar conclusions.[22] The purpose of this presentation is to report the results of studies of alterations in cell membrane transport with shock which do not improve rapidly after treatment.

Methods

Male Holtzman albino rats weighing between 250 and 300 Gm. were used in all studies. They were allowed water, but no food for about 20 hours before the experiments. The animals were anesthetized lightly with ether, and both femoral or subclavian arteries were cannulated with PE-50 tubing. Four hundred units of heparin were injected into each cannula. One of the cannulae was connected to a 1.7 m long PE-50 tubing calibrated in millimeters of mercury for measuring arterial blood pressure, while the other cannula was connected to a 10 ml. glass syringe, which served as a blood reservoir. The duration of anesthesia was about 20 minutes. The animals were restrained and allowed to awaken. Control animals were sacrificed after awakening. Hemorrhagic shock was produced by bleeding into the glass syringe until the mean arterial blood pressure was 40 mm.

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Hg. The maximum amount of blood withdrawn was roughly equal to 3% of the animals body weight, and represented approximately 50% of the animals total blood volume. At about 45 to 60 minutes of shock, the blood pressure began to decrease, and small aliquots of blood were returned to the animal to maintain the pressure of 40 mm. Hg. Three periods of shock were selected for study of the animals.

Group I. Early shock. One-half hour after reaching a pressure of 40 mm. Hg was selected as the time when the maximum amount of blood had been removed to maintain this pressure. Most animals would be expected to survive if the shed blood was returned at this time. The animals in this group were separated into two subgroups: a. Seven animals were sacrificed at that time without treatment; b. Eight animals were treated by return of all the shed blood and a volume of lactated Ringer's solution equal to half the total volume of blood removed and were sacrificed 1 hour later.

Group II. Decompensating shock—the time when 25% of the maximum shed blood had to be returned to maintain a pressure of 40 mm. Hg. Previous studies have indicated that only 40 to 50% of animals will survive if treated at this time. Subgroup a. Ten animals sacrificed at this time without treatment; b. Twenty-two animals treated by return of the remainder of the shed blood and an additional volume of lactated Ringer's solution equal to half the maximum volume of shed blood. These animals were sacrificed 1 or 2 hours after treatment.

Group III. Late or severe shock—the time when 70% of the withdrawn blood had to be returned to maintain a pressure of 40 mm. Hg. Six animals were sacrificed at this time. Treated animals were not studied at this period of shock because they usually died quickly after the remainder of the blood was returned and the early mortality approached 100%.

All animals were sacrificed by rapid decapitation and exsanguination. The liver was removed and transferred to Krebs-Ringer bicarbonate solution (KRB). The composition of KRB was Na+ 142 mEq/L, K^+ 5.8, Mg^{++} 0.6, Ca^{++} 2.6, Cl^- 127.8, HCO_3^- 24, $H_2PO_4^-$ 1.1, SO_4^{--} 0.6, and 11 mM dextrose. Liver slices (0.3–0.5 mm. thick, average weight 50 mg.) were prepared with razor blades within 5–8 minutes after removing the liver. The cation content of some slices was determined immediately after preparation.

Measurement of Membrane Transport. This procedure was done in two ways. Slices (6–10) were incubated for 90 minutes in KRB in an Erlenmeyer flask at 0.5 C with continuous oxygenation with a gas mixture of 5% CO_2 and 95% O_2. Some slices were then removed for analysis. The remaining slices were then incubated with continuous oxygenation at 37 C with slices removed at 15, 30, 45 and 60 minutes for analysis. This method of cooling and rewarming is used to measure the capability of the transport system. Cooling to 0.5 C stops active membrane transport of cations and sodium enters and potassium leaves the cells. The Na content of the total tissue greatly increases and K decreases. With rewarming, the transport system is reactivated and its ability to extrude sodium and reincorporate potassium into the cell can be measured. This capability of membrane transport is demonstrated by total tissue Na decreasing as it is removed from cells and total tissue K increasing as it is reincorporated into cells. In some experiments, liver slices were not chilled. These slices were transferred immediately after sectioning to an Erlenmeyer flask with an oxygenated medium at 37 C. Slices were removed from the flask for analysis after 15, 30, 45 and 60 minutes.

Determination of Cations and Water Content. All slices were blotted by gentle suction through filter paper and weighed. They were dried in a vacuum oven at 95 C for 20 hours and were weighed. The difference between wet and dry weights was the water content of slices. The dry tissue was digested in concentrated, heated HNO_3 to a clear solution. The digestate was diluted and analyzed for Na and K contents, in a Perkin-Elmer atomic absorption spectrophotometer. Cation contents were expressed at mEq/Kg. dry weight. All slices were measured in duplicate and results are expressed as means for the entire group, ± the standard error of the mean (SEM).

Determination of Inulin Space. Triplicate liver slices were incubated in 1 ml. of oxygenated KRB which contained 250 mg. of inulin. Slices were removed after 90 minutes at 0.5 C and after 90 minutes at 0.5 C plus 60 minutes at 37 C. They were blotted and quickly frozen on dry ice. Frozen samples were homogenized in $ZnSO_4-Ba(OH)_2$ mixture and the precipitates separated by centrifugation. Aliquots of clear filtrate were analyzed for inulin by the modified method of Roe *et al.*[19] The ratio of tissue inulin per Gm. wet weight to medium inulin per ml. was equal to the inulin space in per cent of wet weight. It was assumed that the inulin space represented the extracellular space in liver slices. Extracellular cation contents were calculated by multiplying total extracellular water per Kg. wet weight by medium cation concentration. The intracellular cation content was the difference between the total tissue cation and extracellular cation contents. Computation of intracellular water contents, as the difference between total tissue water and extracellular water, allowed expression of intracellular cation concentrations in units of mEq/L.

Results

The cation contents of fresh liver slices are shown in Table 1. The control cation content values per unit dry weight were similar to those reported by Van Ros-

TABLE 1. *Cation Content of Liver Slices from Untreated Animals During Hemorrhagic Shock*

	Na	K
Control (22)	155.73 ± 3.85	362.58 ± 7.31
Shock		
Early—Group Ia(7)	210.80 ± 7.70	329.68 ± 7.18
25% Blood return-Group IIa (10)	266.20 ± 6.28	343.66 ± 6.73
Late—Group III(16)	314.03 ± 21.22	308.34 ± 11.5

± SEM
( ) -Number of animals
Units = Total tissue Na or K content in mEq./Kg. dry weight

TABLE 2. *Cation Content of Liver Slices before and after Treatment of Hemorrhagic Shock*

	Na	K
Control (22)	155.73 ± 3.85	362.58 ± 7.31
Shock		
Group Ia (7) (Before treatment)	210.80 ± 7.7	329.68 ± 7.18
Group Ib (8) (After treatment)	117.18 ± 5.16	353.38 ± 7.52
Group IIa (10) (Before treatment)	266.20 ± 6.28	343.00 ± 6.73
Group IIb (12) (After treatment) (1 hr)	158.25 ± 11.20	362.41 ± 11.04
Group IIb (10) (After treatment) (2 hrs)	142.38 ± 9.41	348.43 ± 10.00

± SEM
( )—Number of animals
Units = Total tissue Na or K content in mEq./Kg. dry weight

sum[28] and Parsons and Van Rossum.[18] Light ether anesthesia and heparinization of the animals did not influence the cation contents of liver slices. There was, however, a progressive increase in Na and a decrease in K in the tissues of animals in shock as compared with unbled controls. The Na content increased nearly two-fold in late shock. The cation content of liver slices before and after treatment is shown in Table 2. The increase in Na with untreated shock was corrected by treatment in both Groups I and II. In Group I b., Na decreased below the control value which would suggest very efficient removal of Na from cells. The decrease in K with shock was also corrected by treatment. Thus, the alterations in cation content in fresh tissue produced by early shock were corrected by simple volume restoration. The results of study of transport capability for animals in Group I at the period of maximum bleed out with chilling of the tissues and rewarming are shown in Table 2 and Figure I. The changes in tissue cation content and water with chilling in tissues from shock animals were parallel to those found in controls. In control animals Na decreased and K increased with incubation at 37 C indicating that active transport was taking place. In animals in shock without treatment there was no transport activity demonstrable. After treatment, however, there was transport activity demonstrable which had returned to the same level of activity as occurred in control animals. Thus, at this period of shock, there was depression of cation transport as measured in this way in animals which were not treated, and this was reversed completely by treatment by volume restoration.

In Table 4 and Figure 2 are shown the results in animals at the period of decompensating shock when 25% of the shed blood was returned. The same depression in membrane transport capability was seen in the untreated animals in shock. With treatment at this period, there was only a minimal return of transport capability 1 hour after treatment. Na did decrease and K increased but the changes were small as compared to the controls.

Table 5 shows the changes with time in slices incubated at 37 C without prior chilling in controls and Group II animals 1 hour after treatment. There is little evidence of active transport in the mean values in this group of treated animals. The small increase in K may indicate some K reaccumulation. In tissues from animals 2 hours after treatment, virtually the same results were found. Na content was 654 (mEq/Kg. dry weight)

TABLE 3. *In Vitro Na-K Transport in Liver in Shock—Group I*

	Na			K		
	Control	B. T.	A. T.	Control	B. T.	A. T.
	(12)	(7)	(8)	(10)	(7)	(8)
Incubation 0.5°C 90 minutes	708.33 ±3.46	655.46 ±17.79	612.54 ±12.89	111.47 ±3.35	100.91 ±1.61	110.85 ±3.8
Incubation 37°C 60 minutes	579.08 ±29.21	660.79 ±20.34	493.57 ±14.63	219.90 ±7.16	117.85 ±6.81	203.13 ±6.15
Difference	−129.2	+5.33	−118.9	+108.5	+16.9	+92.28

Units—Total tissue Na or K content in mEq/Kg. dry weight, ± SEM
( )—Number of animals
B. T.—Before treatment
A. T.—One hour after treatment

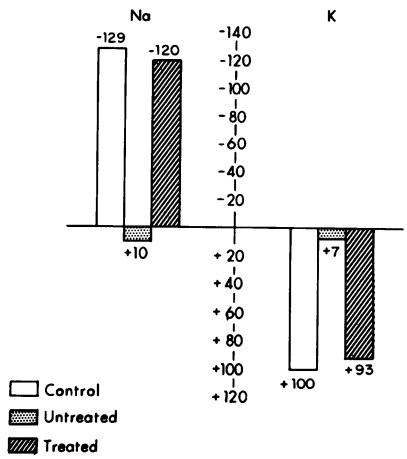

FIG. 1. The transport capability of liver slices from control, untreated and treated animals in early shock is expressed as the mEq./Kg. dry weight of Na extruded (—) and K reincorporated (+) after incubation at 37 C for 60 min. preceded by chilling to 0.5 C for 90 minutes. In untreated shock, Na was + and K was only +7 indicating lack of active transport.

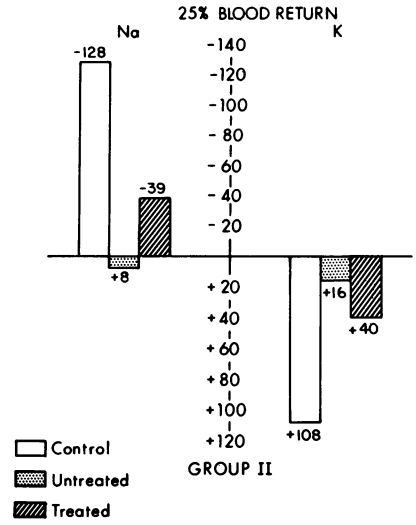

FIG. 2. Plot of the differences in Na and K content of slices at 0.5 C for 90 min. followed by 60 min. at 37 C. Normal transport is present in controls, none with untreated shock animals and minimal transport 1 hour after treatment.

at 15 minutes at 37 C without chilling and 678 at 60 minutes. Na content was 714 after 90 minutes at 0.5 C and 716 after 60 minutes at 37 C. This indicated that there was no active Na transport. The K content increased from 109 at 15 minutes at 37 C without prior chilling to 140 at 60 minutes. With prior chilling, K content increased from 119 after 90 minutes at 0.5 C to 156 after 60 minutes at 37 C. Thus, there was some reaccumulation of K but this was minimal.

Study of the data from individual animals in Group II b. shows some variability from animal to animal, particularly in Na contents. In the 12 animals in Group II b. studied 1 hour after treatment, there was only one animal which showed definite evidence of active transport of both Na and K. In the ten animals in Group II b. studied 2 hours after treatment there were three animals which showed definite active transport of K and some of Na. One of these animals had a satisfactory blood pressure at the time and seemed to be recovering. This animal had evidence of both K and Na transport. A comparison of the data for this animal and another animal in this group which did not demonstrate transport is shown in Table 6.

In Tables 7 and 8 are shown the data on animals in late or severe shock. Not only was there no evidence of active transport in these animals in shock, but Na tended to increase further rather than decrease during incubation. These data also suggest that chilling the tissue from an animal in shock may produce some cold injury. Comparing incubation at 37 C alone (Table 8) with prior incubation at 0 C followed by 37 C (Table 7) indicates that Na increases further when there has been prior cold incubation. When Na reached levels of 850–900 mEq/Kg. dry weight, it was above the medium concentration suggesting that Na was being bound to other molecules inside the cell by altered protein or other mechanisms. Using the measurement of inulin space, the intracellular concentrations of Na and K were calculated in control and late shock animal slices (Fig. 3). Intracellular Na decreased with rewarming from the high level after cooling in normal tissue but continued to increase in shock tissue. This indicates the inability of the shock tissue to extrude Na under these circumstances. The reverse of this occurred with K. The normal tissue could reincorporate K into the cell with rewarming, but the shock tissue could not.

The changes in tissue water in this study were much the same as previously reported by us.[22] Total tissue water

TABLE 4. *In Vitro Na-K Transport in Liver in Shock—Group II*

	Na			K		
	Control	B. T.	A. T.	Control	B. T.	A. T.
	(12)	(10)	(12)	(10)	(10)	(12)
Incubation 0.5°C	708.33	711.80	749.02	111.47	113.90	104.19
90 minutes	±3.46	±33.67	±21.18	±3.35	±2.83	±3.64
Incubation 37°C	579.08	820.20	710.57	219.90	129.70	143.12
60 minutes	±29.21	±29.32	±26.80	±7.16	±3.89	±7.02
Difference	−129.25	+8.40	−39.00	+108.00	±16.00	+40.00

Units—Total tissue Na or K content in mEq./Kg. dry weight, ± SEM
( )—Number of animals
B. T.—Before treatment
A. T.—One hour after treatment

TABLE 5. *In Vitro Na-K Transport in Unchilled Liver Slices Incubated at 37° C after Preparation*

	Na		K	
Incubation Time	Control (11)	Group IIb-one hour after treatment (12)	Control (11)	Group IIb-One hour after treatment (12)
0	131.57 ±5.93	200.27 ±5.77	330.02 ±8.55	330.24 ±5.77
15 min	583.50 ±40.32	691.61 ±18.17	119.53 ±7.12	98.96 ±5.91
30 min	510.19 ±21.12	748.77 ±20.68	154.12 ±10.01	107.34 ±5.39
45 min	466.75 ±16.97	743.91 ±23.05	200.49 ±8.39	110.69 ±6.35
60 min	489.75 ±17.56	756.67 ±26.02	223.79 ±11.16	126.90 ±9.75

( )—Number of animals
Units—Total tissue Na or K content in mEq./Kg. dry weight ± SEM

increased by about 20% in fresh slices in late shock. When the slices were incubated at 0.5 C for 90 minutes, tissue intracellular water increased in both control and late shock slices to 3.52–3.58 with no differences in the two. However, after incubation at 37 C intracellular water decreased significantly in control tissues but did not change in shock animal slices. Thus, the normal cells were able to remove some of the accumulated water but cells after shock could not. The changes in intracellular water with treatment were not measured in the pressent study.

Discussion

The study of Na-K transport in the liver *in vitro* by measurement of tissue slice cation contents after chilling and rewarming in Krebs-Ringer's solution has been reported by a number of investigatiors.[13,28] It has been shown that during chilling of slices at 0–1 C, suppression of cellular metabolism produces complete cessation of the energy-linked cation transport functions.[13] After chilling of tissues for 90 minutes, Na^+ and Cl^- diffused from the medium into tissue water to establish a Gibbs-Donnan type equilibrium.[13] Potassium diffuses out of tissue water, but the ratio of [K] in slices/ [K] in the medium exceeds by a factor of 4–5 the similarly estimated ratio for the diffusable anion Cl^-. This behavior of K was thought by Heckman and Parsons to indicate the presence of this cation in the tissue water in a bound and free form, the bound K being responsible for deviation of K distribution from the Gibbs-Donnan equilibrium. Upon rewarming of tissues at 37 C, a reactivation of metabolically linked cation transport has been shown.[16] It is known that intracellular Na would decrease against a concentration gradient and also against an electrical gradient as it has been shown that the rat-liver cell interior is negative to the outside.[29] The reaccumulation of K by liver slices following incubation at 37 C also occurs against an electro-chemical gradient as the K equilibrium potential calculated using the estimated intracellular K and the medium K in the Nernst equation exceeds the measured transmembrane potential in liver (−43 mv).[29] These criteria fulfill the definition of Ussing of active transport.[27] Inhibition by the cardiac glycoside ouabain of active cation movement in rat-liver slices has been shown at a concentration of .75 mM and supports the "active" nature of cation movements in tissue slices. With the same methodology as used in the present study, we have previously found that ouabain did, indeed, inhibit active cation movements against gradients in liver slices from control unbled animals. Total cation content values per unit dry weight in the present study were similar to those reported by Van Rossum.[28] There was no obvious effect of the prior use of light ether anesthesia and heparinization of the animals in this study.

In the present study, we found in early shock, within a half-hour after reaching an arterial blood pressure of 40 mm. Hg, that the transport capabilities of liver slices from such animals were severely impaired. No evidence of active transport was demonstrated in these tissues following cooling and rewarming. This was true for both

TABLE 6. *Comparison of Na and K Transport in Two Animals, Two Hours after Treatment of Shock at 25% Blood Return—Group IIb*

	Na		K	
Time	Animal A	Animal B	Animal A	Animal B
Fresh	96	108	323	444
0.5°C for 90 min	623	775	123	120
37°C for 15 min	567	719	137	109
30 min	527	836	179	116
45 min	508	829	230	146
60 min	537	802	223	140

Units = Total tissue Na or K content in mEq./Kg. dry weight

TABLE 7. *In Vitro Na and K Transport in Late Shock (Group III)*

		Chilled	Rewarmed—37°			
	Fresh slices	0.5°-90 min	15 min	30 min	45 min	60 min
K Control (6)	335.25 ±7.21	95.72 ±3.61	109.74 ±6.80	165.04 ±9.66	201.79 ±11.18	215.10 ±10.07
K Shock (6)	311 ±17	99 ±7	94 ±5	123 ±5	114 ±2	113 ±4
Na Control (6)	140.89 ±7.58	753.87 ±23.69	683.22 ±46.05	569.80 ±38.30	517.92 ±44.37	606.04 ±28.62
Na Shock (6)	288 ±26	739 ±30	787 ±42	862 ±59	915 ±48	927 ±39

Units = Total tissue Na or K content in mEq./Kg. dry weight ± SEM
( ) = Number of animals

Na and K. When animals were treated by volume replacement at this early period of shock, however, the transport capabilities of such tissues returned to normal within 1 hour. In Shires' studies in which he and his group measured the transmembrane potential of skeletal muscle of animals in shock,[3,5,24,26] the transmembrane potential did not change until the animal had been in shock for 1 to 1½ hours. Following this decrease in membrane potential, transport capability was found to be reduced as determined by tissue biopsy, cation contents and the chloride space calculated using the Nernst equation. The changes in the liver in our study took place much more rapidly than in Shires' study of skeletal muscle. In preliminary studies, we have found that the transmembrane potential in the liver which normally is −40 to −43 mv decreased very rapidly within the first half-hour after producing shock. Shires has also found this to be true. This rapid change in membrane potential is in agreement with the rapid impairment of transport capability measured *in vitro* in the present studies. Reasons for this rapid impairment in liver cell membrane transport with shock could be that liver blood supply is more severely impaired than is skeletal muscle, or that in the resting animal the metabolic needs of the liver are greater than skeletal muscle. In animals in the middle period of shock after 25% blood return, when the circulation was beginning to decompensate by vasodilatation requiring blood to be returned to the animal, the findings were similar. There was little or no evidence of transport of the tissue slices from the animal in shock. The question arises as to whether chilling, which has been used to stop active transport in this study, could have a detrimental effect upon a tissue which is already injured by shock. The possibility that this may occur was studied by incubating some slices at 37 C without prior chilling. In prior studies, we found that such slices from animals in shock were capable of some active transport, but this was less than 50% of the control values. In the present study, comparing Tables 4 and 5 it is seen that when the slices were chilled initially and then rewarmed, there was a further increase in Na rather than a decrease and a small increase in K during the period of rewarming. When, however, the tissue was not chilled, but kept at 37 C there was an initial, large increase in Na at 15 minutes

TABLE 8. *In Vitro Na and K Transport in Late Shock in Unchilled Liver Slices Incubated at 37 C*

	Na		K	
Incubation Time	Control (11)	Late Shock (6)	Control (11)	Late Shock (6)
0 Fresh slices	131.57 ±5.93	314 ±21	330.02 ±8.55	308 ±11
15 min	583.50 ±40.32	796 ±31	119.53 ±7.12	95 ±5
30 min	510.19 ±21.12	871 ±43	154.12 ±10.01	119 ±7
45 min	466.75 ±16.97	784 ±48	200.49 ±8.39	126 ±4
60 min	489.75 ±17.56	758 ±37	223.79 ±11.16	127 ±15

( ) = Number of animals
Units = Total tissue Na or K content in mEq./Kg. dry weight ± S.E.M.

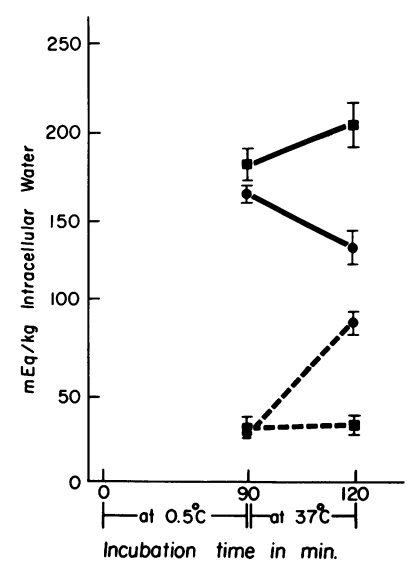

FIG. 3. Plot of intracellular concentrations of Na and K in mEq./Kg. intracellular water at 90 min. at 0.5 C and after 37 C for 60 min. in slices from control animals and animals in late shock.

●———● Na—control
■———■ Na—shock
● — — ● K—control
■ — — ■ K—shock

and a decrease in K comparable to controls, but then these values leveled off without any further increase in Na and some increase in K. This would indicate that some residual Na-K transport capability was present in liver slices from severely shocked animals, but that this activity could not be retained during chilling. Thus, lowered synthesis of energy during cold incubation which only reversibily suppressed Na-K transport capability in control liver slices may have produced a more complete breakdown of this transport mechanism in late shock animal tissues. Thus, the Na-K transport mechanism is not only depressed in shock tissues but could also be more susceptible to any further metabolic stress which reduces synthesis of energy rich compounds. Further injury with chilling, if it did occur, did not seem to be severe because, when early shock animals were treated, transport capability returned promptly to normal even when the tissue was chilled.

When animals were treated by volume replacement at the period of decompensating shock when 25% of the blood had to be returned to maintain a blood pressure of 40 mm. Hg, there was little or no evidence of improvement in transport capability in the tissues from these animals. In looking at the data from individual animals, however, it was seen that in one of the 12 animals sacrificed 1 hour after treatment there was definite evidence of transport even though this was not evident in the mean values for the entire group. In the animals from group II sacrificed after 2 hours there was improvement and evidence of transport capability in three of ten animals. An example of this is shown in Table 6 in which animal "A" showed definite evidence of transport capability by extrusion of sodium and incorporation of potassium whereas animal "B" showed no evidence of transport capability. Thus, it seems that after treatment of prolonged shock with decompensation, there is variability among the animals and some showed improvement in transport capability whereas others did not. With this period of shock and treatment, survival would be predicted to be only 40–50%. Although improvement in transport capability could be related to eventual survival, we do not have sufficient information or experience at this time to speculate about this. This method of measurement of transport capability requires sacrifice of the animal, and does not allow documentation of survival in the same animal. Further studies are contemplated at longer periods of time after treatment to see if transport capability returns in a larger number of the animals. Several of the animals in this series that did have return of transport capability also had a very satisfactory blood pressure at the time and seemed to be responding satisfactorily. However, one animal which demonstrated return of transport capability had a blood pressure of 60 mm. Hg at the time of sacrifice. The failure of early return of transport capability in many animals after treatment could be due to a progressive alteration or damage to the cell membrane with shock.

In late shock, transport capability was greatly depressed and in tissue slices from these animals, with chilling and rewarming, Na continued to increase and K inremained virtually unchanged. When tissue slices from animals in late shock were incubated without prior cooling, Na did not progressively increase but remained fairly stable and K increased slightly. This indicated virtually no transport capability. Treatment of these animals in late shock was not carried out in the present study because we have found that even with treatment, the survival time was short, very few of the animals improved even transiently, and the mortality was virtually 100%.

The large variability in Na contents of the tissue slices is evident. This is always a problem in studying tissue slices because of several technical factors. Sodium is in the incubating medium in a concentration of 142 mEq/L and the slices must be blotted before weighing and preparation. Slight variations in blotting could produce large changes in content. This probably accounts for much of the variability in Na contents. Also, the liver injury with shock probably varies in different animals and could increase the variability of Na contents. With K, more constant values are obtained. For these reasons we are hesitant to conclude that there is active membrane transport present in tissue slices unless the amount of Na extruded is 100 mEq/Kg. dry weight or more, and the K reincorporated into the cell is 30–50 mEq/Kg. dry weight or more. The sensitivity of the transport system to injury is evident from the values in tissues removed from an animal, with slices prepared and incubated in a satisfactory environment at 37 C. Even in normal tissue there was a large increase in Na content at 15 minutes and a decrease in K, indicating that the period of preparation of the slice and initial incubation produced a tremendous alteration in these cations. In the normal tissue, however, once placed in this satisfactory environment, transport capability returned and was demonstrable. In addition, it can be seen in the kinetic studies of cation contents as a function of incubation time in shock tissues, that there was a tendency toward deterioration of the tissue after 1 hour of incubation at 37 C. Often the last value at 60 minutes showed an increase in Na rather than a progressive decrease if transport capability was maintained. This tail-end change may be a problem of deterioration of the tissue *in vitro*.

We have previously demonstrated that ATP levels were greatly reduced in early and late shock in liver tissue.[4] The failure of Na-K transport in shock might be related to such a reduction of ATP levels in the cells, but there

is also the possibility that Na-K transport failure is a primary event due to an alteration in the cell membrane and ATP synthesis is effected secondarily. A decrease in oxidative metabolism due to changes in transport and/or an effect of transport related changes in the cytoplasmic cationic milieu on cellular respiration are further possibilities. A detrimental effect of Na on mitochondrial respiratory control has been shown by Gomez-Puyou *et al.*[9]

The cation contents in fresh liver slices from untreated animals in shock showed a progressive increase in Na with time reaching twice the control value in late shock. With this, there was a concomitant decrease in K of about 15 percent. The methodology of this study does not allow measurement of intracellular and extracellular distribution of these cations in the fresh tissue slice. Thus, it cannot be stated with certainty that these changes represent changes in intracellular concentration. The question can be asked whether the increase in Na in the fresh tissue slice of the shocked animal could be due to increased blood or fluid in the liver with shock. The slices were prepared, of course, from the livers of both control and late shock animals which were exsanguinated prior to excising the livers. In control animals, exsanguination has been shown to leave a residual quantity of blood to the extent of no more than 4% of liver weight. Secondly, slices from both control and shock animals were blotted by suction through filter paper. Thus, sequestration of large amounts of blood or fluid seems unlikely to account for this difference. However, even if it was assumed that all of the increase in tissue water found in late shock slices was due to plasma from sequestered blood, or from increased extracellular fluid, this would not explain the increased Na. The increase in total tissue water with shock was approximately 500 Gm/Kg. dry tissue. Assuming that this was all due to pooled plasma and that the Na concentration of plasma was 140–160 mEq./L, Na could be increased about 70–80 mEq/Kg. of dry tissue. The average increase in Na with late shock was about 160 mEq/Kg. dry tissue. In order to produce this increase in Na, the amount of residual blood in the liver would have to be about 50% of liver weight which does not seem possible. Shires and his group in their study of muscle cation content of baboons in shock with the calculation of intracellular and extracellular distribution by membrane potential, and chloride space measurements, found an increase in intracellular Na of about the same magnitude as we found in total content in liver and about the same decrease in intracellular K. The fit of our data with his suggests that the sequential increase in Na in the liver with shock was indeed due to intracellular accumulation. Shires, however, found no change in total cation content of Na or K in muscle, in contrast to our study of the liver. This is most likely due to differences between liver and muscle. Following treatment, the Na and K contents of the tissues returned toward normal or were even better than normal, suggesting that treatment reversed the transport abnormality to a reasonable extent *in vivo*. Again, however, it was not possible to calculate the intracellular concentrations of these cations in the fresh tissue. The greater change in Na than in K could indicate a specific permeability defect for Na in shock which was associated with an increase in tissue water. The *in vivo* measurement of transmembrane potentials in skeletal muscle in shock by Shires group has suggested alterations in the Na pump and/or increased Na permeability. This is compatible with the data of the present study. An earlier finding of increased Na+K-ATPase activity in the liver with hemorrhagic shock[27] may be related to the increase in tissue Na levels in shock, as this cation would stimulate the transport enzyme activity. The Na related increase in transport enzyme activity may not indicate necessarily an increased level of active cation translocation across the cell membrane, but a direct Na effect on enzyme activity. The coupling between Na activation of the transport enzyme and the transmembrane cation translocation would require a sufficient amount of the high-energy compound ATP. This, of course, has been found to be severely reduced in hemorrhagic shock in studies from our laboratory.[4] Flear *et al.* have measured similar changes in skeletal muscle of increased water, Na and Cl and decreased K in patients during major operations.[8]

When the intracellular and extracellular components of tissue Na were estimated by measurement of the inulin space, it was found that there was a significant increase in intracellular sodium in shock with chilling compared with chilled control rat-liver slices. The ratio of intracellular to medium Na in control liver slices was 1.15 which is similar to that shown by Heckman and Parsons.[13] This led these authors to conclude that there is a slight modification from the Donnan equilibrium for Na. This ratio was significantly higher in late shock animal liver slices (1.50) and may indicate a further deviation from Donnan equilibrium. However, a definite conclusion on this point cannot be made as diffusible anions were not measured in this study. With rewarming of late shock animal liver slices, Na contents continued to increase both in total tissue and in intracellular water indicating a complete failure of active Na extrusion. This increase in intracellular Na in late shock would increase the ratio of Na in tissue water to medium over that observed with chilling alone. This again might be due to deviation of the Na distribution from that expected on the basis of Donnan equilibrium in the liver slices of shocked animals. It is possible that the

increase in Na represents Na that becomes bound or immobile in some other manner within the cell. Along with the Na increase there was a progressive increase in water contents with warming of shocked animal liver slices. This may represent a breakdown of the energy-linked Na transport process, allowing Na to accumulate intracellularly with subsequent increase in diffusable anions and water as was found during cold incubation by Heckman and Parsons.[13] The increase in Na intracellularly with warming was associated with the failure of K reaccumulation.

Although not specifically studied in this present report, we have previously found that total tissue water during shock increased from 2.3 Kg. water/Kg. of dry tissue in controls to 2.8 Kg. water/Kg. of dry tissue with late shock. After cooling, the water content of both control and shock tissue slices had increased to 3.52 and 3.58 which were no different. Intracellular water had increased in both. Thus, with cessation of active transport in the control and shock tissues by cooling, the increase in water intracellularly was comparable in both. With incubation, however, at 60 minutes at 37 C the intracellular water content from rat-liver slices in control animals decreased from 3.52 to 2.54, a significant decrease; whereas, in shock tissues the change was from 3.58 to 3.43 which was not a significant change. Thus, as active transport was initiated by rewarming, the control or normal tissue was able to decrease its water content significantly, presumably by extruding Na and therefore a decrease in water; whereas, the shock tissue could not do this. In addition, the extracellular water of tissue slices in control animals did not change significantly with rewarming, but in shock tissues with rewarming there was an increase in extracellular water as well. The increase in cell water with the Na-K transport breakdown in shock may represent the cell swelling which has been observed in shock,[7,14] and in ischemia.[25] De Palma *et al.* have now shown that the cell swelling seen by electron microscopy with shock can be corrected by treatment.[7] Although a derangement in the Na-K transport system seems to be a reasonable explanation of cell swelling in shock, other possibilities cannot be excluded. These include 1) breakdown and accumulation of tissue metabolites which increase osmotic concentration, thus promoting the entry of water intracellularly; 2) breakdown of an active water transport mechanism following energy depletion. In chilled rat-liver slices the breakdown of the Na-K transport mechanism has been shown by Heckman and Parsons[13] to be the most important factor in swelling, at least during the first 1 to 2 hours of cold incubation. Although the data presented in this paper, and in our previous work do not show a direct relationship to the Na-K transport failure and changes in cell water contents and, thus, cell volume, to altered oxidative metabolism of mitochondria such a relationship seems likely in shock. In liver slices, the rate of respiration is reduced when the active transport of Na and K is inhibited by the cardiac glycoside, ouabain. This has been interpreted as being due to a coupling between cation transport and respiration by ADP released in the cation transport process. The ouabain sensitive portion of respiration has been considered to represent the energy requirements of cation transport. In our previous study, we found a 50% decrease in respiration of liver slices in shock when the slices were previously chilled. This decrease could be in part due to a decrease in the Na-K transport process. If there was no uncoupling of mitochondrial respiration, the reduced oxygen consumption would be considered more than sufficient to sustain the transport process. But since partial uncoupling of mitochondrial oxidative phosphorylation has been shown in shock by us and others,[1,17,21] this decrease could be related to a primary or a secondary failure of Na-K transport. A decrease in respiration was also found in late shock animal liver slices which had not been chilled and this decreased respiration in late shock was not altered by ouabain at a concentration of 0.5 or 1 mmoles/L. The absence of the ouabain inhibitable portion of respiration with shock also supports the finding of failure of Na-K transport in shock. A further role of altered Na-K transport in shock is suggested by the knowledge that ion transport is coupled to active sugar and amino acid transport in the intestine and sugar transport in skeletal muscle. Thus, alterations in the transport system with shock could influence many cell functions.

Summary and Conclusions

1. *In vitro* cell membrane transport of Na and K in the liver was severely impaired during hemorrhagic shock.
2. This defect was rapidly corrected in early shock by nonspecific treatment with volume replacement.
3. As shock continued, the depression of cell membrane transport was not rapidly corrected by treatment and persisted.
4. Other alterations in hepatic cell function produced by shock which we have measured have been rapidly corrected by treatment.
5. Persistent depression of cell membrane transport may be a limiting factor in severe, prolonged shock.

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Discussion

Dr. G. Thomas Shires (Dallas): We presented, as Dr. Baue mentioned, to this Association last year some studies in parallel using *in vivo* technics which I would like to recount for a moment and ask Dr. Baue one question.

The studies presented here last year utilized the direct measurement of skeletal muscle transmembrane potential in hemorrhagic shock in intact primates. The results indicated the existence of a major alteration in membrane function in skeletal muscle during prolonged hemorrhagic shock. These studies also showed the reversibility of the cellular changes following resuscitation, with survival of the primate.

Subsequent studies that we recently reported at the Society of University Surgeons measuring action potentials in conjunction with resting potentials were shown during the control and the shock and resuscitation phases. We did this to get a better handle on the *in vivo* movement of ions.

Utilizing the transmembrane potential and muscle biopsy in the previous study, we demonstrated the movement of sodium, chloride and water into the muscle cells accompanied by a loss of muscle cellular potassium in the living primate in hemorrhagic shock.

This last slide, from these most recent studies shows a decrease in the amplitude of muscle action potentials as well as prolongation of both the repolarization and depolarization times in hemorrhagic shock. Resuscitation reversed these changes acutely except for the repolarization time which recovered more slowly.

These changes then confirm, *in vivo*, the previously recorded increase in intracellular sodium and decrease in intracellular potassium. While intracellular sodium returned to normal levels very promptly with therapy, the prolonged repolarization seen here gives, *in vivo* in recoverable primate shock evidence that there may well be a delay in recovery in some cells, particularly in the potassium repletion phase.

I might ask Dr. Baue one question and that is, do you believe the transport defects which you measure, which agree with our own *in vivo* whole cell studies, might be slower or impossible