Shock Reactions in Skeletal Muscle. III.:

The Electrolyte Content of Tissue Fluid and Blood Plasma before and after Induced Hemorrhagic Shock

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A PROPER knowledge of the pathologic and physiologic reactions of single cells during hemorrhagic shock is necessary for the evaluation of correct therapeutic measures. The skeletal muscle cell is of great importance to the electrolyte equilibrium, especially with regard to potassium, as muscular tissue constitutes about 40% to 50% of the cellular tissue of the body. It is known that there is an increase in the potassium content of plasma following mechanical trauma to muscular tissue, impairment of circulation by tourniquet application, or arterial occlusion. (For literature survey see Rosenthal and Millican,[20] Millican.[18].)

A similar increase of plasma potassium had been reported by Kerr [16] and Thaler [23] after hemorrhage, and Baetjer [1] stated that a decrease of the rate in blood flow of 80% was necessary before any marked increase of plasma potassium concentration appeared. The elevated plasma potassium concentration is partly due to a release of skeletal muscle potassium; this has been confirmed by analysis of uninjured muscle samples before and after hemorrhagic shock, from rabbits [17] and dogs.[13]

In a previous report [13] a marked discrepancy was observed between the decrease of potassium content after hemorrhagic shock in large muscle biopsies analyzed directly, and between single muscle cells and muscle fiber bundles dissected out in Krebs-Ringer solution before the analysis. In the former case the loss of potassium from the tissue was 2 to 3% of the pre-hemorrhagic value while in the latter there was a mean loss of 26% (10–50%). This discrepancy raised the question whether the profound loss of potassium from single skeletal muscle cells and muscle fiber bundles reflected an *in vivo* leakage of potassium from single cells into the interstitial fluid. In that case this *in vivo* leakage would be included in the electrolyte analyses of large muscle pieces while in the case of single cells the high tissue fluid potassium concentration would be washed out into the dissection solution.

From the analysis of the dissection solution it would not be possible to evaluate such a wash-out as there is always a leakage of potassium from the cut ends of the muscle biopsy and from damaged muscle fibers.

In the present investigation tissue fluid has been sampled before and after induced hemorrhagic shock and the sodium and potassium content of this fluid has been determined and compared to plasma electrolyte values.

Material and Methods

Eight dogs of mixed breed and of both sexes were used for the investigation. All the dogs were kept on the same diet for at least 2 weeks before the day of the experiment.

The dogs were anesthetized with intravenously administered Nembutal (Abbott) 30–40 mg./Kg. One of the carotid arteries
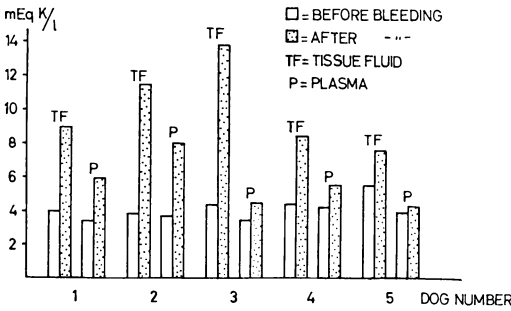
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FIG. 1. (See text.)

was exposed and a polyethylene tube connected to a Y-tube was inserted. One of the Y-tube limbs was connected to a pressure transducer and recorder and the other to a bleeding cup adjusted in such a way, that the system automatically maintained a predetermined blood pressure level in the dog. Heparin (2.5 mg./Kg.) was given intravenously in order to render the connections patent. At the beginning of the experiments the blood pressure of the dogs was 140–180 mm. Hg.

Hemorrhagic shock was induced by bleeding the dogs to a blood pressure of 45 mm. Hg; this blood pressure was maintained for 135 minutes. To determine the percentage of the original blood volume, that was removed during bleeding, ^{131}I-albumin was given before the bleeding.

Sampling and electrolyte analysis of tissue fluid before and after the hemorrhage was performed according to the method described by Haljamäe.[14]

The hair from an area over the thigh muscles was removed and the skin surface cleaned and covered with sterile liquid paraffin. Under a binocular operating microscope a small incision 3–5 mm. was made through the epidermis and the cutis and subcutis was transected by atraumatic dissection taking advantage of subcutaneous connective tissue membranes. Damage to vessels was avoided. The incised cavity was kept filled with liquid paraffin to prevent evaporation and the mouths of the pipettes used for sampling were sealed in so that the original electrolyte concentration could not change. With this technic it was possible to obtain minute quantities of tissue fluid (25–30 nl, 1 nl $= 10^{-9}$) from the outside of the muscle fascia by applying a light pressure below the pipette.

When removing the pipette from the cavity the lower end was also sealed with liquid paraffin. The sample was placed on a quartz glass under liquid paraffin and the few cells of the fluid were allowed to sediment. The proteins were coagulated, and duplicate analyses were made for potassium and sodium using an ultra-micro flame photometer.[15] In this way it was possible to analyze 0.5×10^{-12} Gm. of potassium and sodium per nl with an accuracy ± 0.03.

Arterial blood samples were taken before the bleeding and after 135 minutes at 45 mm. Hg and transferred into siliconed tubes under liquid paraffin. The blood was immediately centrifuged at room temperature [3] and microsamples of the plasma were taken from beneath the paraffin cover with sealed pipettes. The microsamples of plasma were applied onto the same quartz glass as the tissue fluid samples and the same photometric analysis was performed to avoid discrepancies caused by different analytical procedures.

To estimate changes of the fluid content of the muscle tissue during shock, triplicate samples of 0.05–0.1 Gm. of muscle tissue were taken before and after the bleeding period. These pieces of muscle were immediately weighed repeatedly with careful noting of elapsed time from the moment of biopsy to each weighing. In this way the probable *in vivo* weight at time zero could be extrapolated. The dry weights of the muscle pieces were obtained after drying over night at 100° C. to constant weight.

Results

The potassium and sodium concentrations of tissue fluid and plasma, from five dogs, before and after the hemorrhagic

shock period are presented in Figures 1 and 2.

Tissue fluid concentrations given before bleeding are the mean values of duplicate analyses of three different samples. After 135 minutes at a blood pressure of 45 mm. Hg it was much more difficult to sample tissue fluid, because of redistribution of fluid from the subcutaneous tissue to the intravascular compartment. Hence the values given represent duplicate analyses of the sample since it was impossible to obtain sufficient amount (> 10 nl) of tissue fluid for three analyses.

As can be seen from Figure 1 there was a marked increase of the plasma potassium concentration in all dogs after induced hemorrhagic shock amounting to a mean value of 1.95 mEq./l. However, the potassium concentration changes of tissue fluid were much greater, amounting to a mean value of 5.50 mEq./l. The sodium concentration of plasma decreased in dogs 1 and 5, and did not significantly change in dog number 4. In dog number 3 no values were obtainable due to contamination during analysis. The changes in electrolyte concentrations also correlated with individual reactions in each experimental animal as there were great discrepancies between the per cent of initial blood volume removed in order to maintain a blood pressure of 45 mm. Hg.

From Figure 3 it can be seen that the per cent increase of tissue fluid potassium concentration was linear to blood volume depletion. The same was true with plasma potassium concentration increase, with the exception of dog 3, which was depleted of 65% of its initial blood volume. In this case the exchange between the tissue compartment and vascular compartment was too impaired to wash out the increased tissue fluid potassium. The analysis of water content of skeletal muscle pieces, where *in vivo* weight and absolute dry weights were extrapolated, showed no marked change or a slight increase of water content after
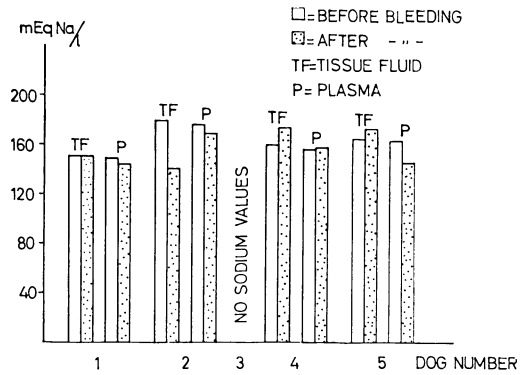

FIG. 2. (See text.)

hemorrhagic shock. However, the increase was, insignificant ($P < 0.5$) and suggests a slight degree of cellular edema arising during the shock.

Discussion

The induction of hemorrhagic shock by depletion of 40–65% of the initial circulating blood volume produces a combination of several circulatory, metabolic and hormonal derangements of physiological processes. The peripheral circulation of skeletal muscle and skin is markedly reduced, resulting in inadequate perfusion and inadequate exchange between the vascular bed and the tissue fluid, as indicated by the decreased central lymph flow during hemorrhagic shock.[24] This decreased exchange appears to be due to the combined effects of lowered hydrostatic pressure, reduction of filtrable capillary surface owing to peripheral vasoconstriction, and reduction of the oncotic pressure in the vascular compartment resulting from protein depletion and plasma dilution by tissue fluid. These changes bring about an interference with the oxidative metabolic activity of the skeletal muscle cells and during the ensuing hypoxia, the muscle cells seem no longer capable of sufficient energy production to maintain the high intracellular potassium concentration and inhibit sodium influx.[5, 8, 12, 13, 17, 19]

Due to the decreased exchange between tissue fluid and the capillaries the potas-
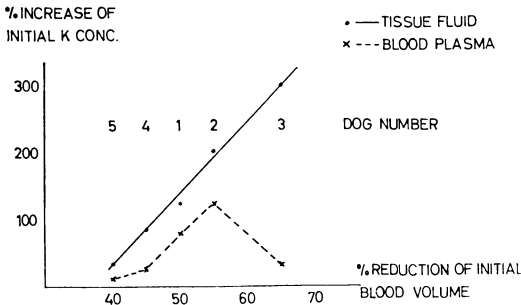
FIG. 3. (See text.)

sium that leaks out of skeletal muscle cells during the hypoxia of hemorrhagic shock seems mainly to remain in the tissue fluid compartment with only a slight exchange to circulating blood thus creating great discrepancies of measured potassium distribution. The sampling of tissue fluid outside the muscle fascia in the present investigation probably does not reveal the absolute concentrations in tissue fluid within the muscle but only indicates the diffusion of potassium across fascia from a fluid which is richer in potassium. If no equilibration between the potassium concentrations in these two compartments of tissue fluid takes place, it might be possible that the skeletal muscle tissue fluid concentration of potassium is even higher than observed with this indirect method.

There seems, however, to be a direct correlation between the percentage of initial blood volume depletion and the potassium concentration increase of the subcutaneous tissue fluid. This is also true in the case of the increase of blood plasma potassium to a certain point. The analysis of blood plasma thus reveals part of the tissue reaction caused by hemorrhage amounting to 40–55% of initial blood volume. On the other hand, when the blood volume exceeds these values, plasma analysis gives a wrong impression of conditions in cellular tissue.

These discrepancies between potassium concentrations of tissue fluid and plasma are also of interest considering the toxicity of potassium. Zwemer and Scudder[25] con-

sidered potassium a "toxic factor" in shock as there was an increase of blood potassium. However, later it was considered that the potassium increase of plasma in traumatic shock was too small to be considered *toxic* when compared to the high values necessary for toxicity in normal animals infused with potassium solution (for literature survey, see Millican[20]).

Apply Nernst's equation one obtains:

$$E = R \ T/F \ ln. \ (K)_i/(K)_e.$$

E = potential difference   R = gas constant   T = absolute temperature   F = Faraday

where K_i and K_e represent the intra- and extra-cellular potassium concentrations, respectively.
(Normally $(K)_i \approx 120$ mEq./l.; $(K)_e \approx 4$ mEq./l., which gives $(K)_i/(K)_e \approx 30$.

When infusion of a potassium solution is undertaken, a large quantity of potassium in the plasma is necessary before the increase of the surrounding tissue fluid potassium is high enough to give a considerable lowering of the quotient $(K)_i/(K)_e$. After hemorrhagic shock, however, there is a mean loss of 26% of the intracellular potassium[13] most of which will apparently be found in the tissue fluid with only minimal exchange to blood plasma. This means that $(K)_i \approx 110$–60 mEq./l. and $(K)_e \approx 7.5$ – 13.5 mEq./l. giving $(K)_i/(K)_e \approx 15 - 4$, indicative of profound impairment to cellular activity and excitability. A similar analysis could partially explain the increased sensitivity of shocked animals to potassium intoxication.[22]

The increase of plasma potassium is accompanied by a lowering of plasma sodium as would be expected from a decrease of cellular oxygen supply with insufficiency of cellular metabolism. A similar elevation of tissue fluid sodium was observed in only one experiment, while the others showed slight increase or no significant change.

Other evidence of cellular damage is increased water content of skeletal muscle

after hemorrhagic shock, since influx of water together with translocation of electrolytes is an early indication of cellular damage.[6] If there were no cellular edema a loss of water would be expected since the vascular bed of skeletal muscle is decreased, followed by a compensatory redistribution of tissue fluid.[10, 21] Dunn et al.,[11] found that 67 per cent of the plasma dilution of tissue fluid is derived from the stomach, intestine and skin.

In these experiments the disappearance of tissue fluid from the subcutaneous tissue was also noticed indirectly because of the difficulty of obtaining enough fluid for analysis after the hemorrhagic shock period. The unaltered or slightly increased ($P < 0.5$) water content of the entire skeletal muscle indicates an increase of the intracellular water content. From a clinical point of view it may be assumed that analyses of serum or plasma samples for electrolytes do not always provide information on the actual state of electrolyte equilibrium of cellular tissues.

The combination of plasma and skeletal muscle biopsy analyses [17] gives an erroneous impression of the cell reaction since potassium that leaks from the intracellular compartment into the tissue fluid is included in the analysis of the muscle sample. It is likely that the reaction to shock in man follows the same electrolyte patterns as observed in the dog. Dogs are more suitable for the registration of more exact potassium changes as slight degrees of potassium leakage from erythrocytes in the preparation of plasma samples or in vivo measurements do not interfere with the experiment as the main electrolyte of dog erythrocytes is sodium.[3, 4, 7] Even if a correction is made for the extracellular fluid content of the muscle by assuming that this concentration is equivalent to that in plasma, an excessively high intracellular concentration is estimated. The loss of potassium, measured in this way, amounts to only slightly more than that exchanged to

plasma, while most of the high tissue fluid potassium is considered intracellular. That such profound leakage from the intracellular space to tissue fluid occurs may be of clinical importance both for diagnostic and therapeutic purposes.

Replacement of the lost plasma volume will wash out the high potassium of tissue fluid as there is a markedly increased circulation of lymph and tissue fluid after retransfusion.[24] This will lead to an increase of the $(K)_i/(K)_e$ quotient, with at least theoretical improvement of the cellular potential as the high potassium concentration of tissue fluid will be diluted by plasma with lower potassium concentration. The most important aspect for survival, however, is redistribution of potassium into the intracellular compartment and improving oxygen supply to the cells.

Summary

Hemorrhagic shock was induced in dogs by bleeding to an arterial blood pressure of 45 mm. Hg in the carotid artery. The initial blood volume was determined by ^{131}I-albumin distribution. Tissue fluid samples and blood plasma samples were taken before the bleeding and after 135 minutes at a blood pressure of 45 mm. Hg. Potassium and sodium analyses were performed with the aid of an ultra-micro flame photometer.

Following induced hemorrhage a marked rise in tissue fluid potassium concentration (30–300% of initial conc.) but only slight corresponding increase of plasma potassium concentration (10–120% of initial conc.) were observed. There was a linear correlation between the increase in per cent of the initial potassium concentration of tissue fluid and reduction in per cent of the initial blood volume. This suggests a tissue fluid potassium increase resulting from leakage of intracellular potassium into the surrounding tissue fluid. The discrepancies between tissue fluid and plasma potassium concentrations seemed to be due to

impaired blood supply and decreased fluid exchange between these two compartments.

There was a decrease of the plasma sodium concentration after the shock period indicating sodium leakage into the intracellular compartment but the sodium concentration of the tissue fluid varied. The water content of muscle samples taken before and after the hemorrhagic shock period showed no change or only slight increase ($p < 0.5$) despite the reduced vascular bed and tissue fluid distribution to the intravascular compartment. The results demonstrate that for evaluation of electrolyte distribution and actual metabolic conditions of cellular tissue during shock, electrolyte analyses of plasma samples alone are insufficient.

Acknowledgment

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