density in skeletal muscle of the dog is higher than in skeletal muscle of the rabbit. This difference could be explained by the greater physical activity of the dog. The influence of physical activity on capillary density was shown by Petren *et al*(5) in skeletal and heart muscle in trained and untrained guinea pigs. Also Wachtlová *et al*(6) found a significant higher capillary density in the myocardium of hares than in the myocardium of rabbits of the same age and body weight. This suggests that capillary density of muscles is influenced by the work load imposed on the heart by physical activity.

The myocardium of the domestic rabbit shows a significantly higher capillary density than the myocardium of the dog, despite the greater physical activity of the dog, which is reflected in the higher capillary density in skeletal muscles. This demonstrates that in heart muscle the higher metabolic rate in smaller animals has probably a greater influence on capillary growth than physical activity. In skeletal muscle, however, the effect of physical activity seems to be more important than the effect of the metabolic rate.

Regional differences of capillary density in the heart and differences between gastrocnemius and vastus muscles remain the same despite the variations in the absolute values.

Further investigations regarding the significance of other factors influencing capillary growth are required.

*Summary.* The capacity of the terminal vascular bed as an indicator of the capillary density in heart and skeletal muscles of the rabbit and the dog was investigated. Tissue samples were taken from two regions of the heart muscle (apex and septum) and from two different skeletal muscles (gastrocnemius and vastus lateralis). The capillary density in heart muscle was higher in the rabbit than in the dog. On the other hand, the capillary density of skeletal muscle was greater in the dog. All differences were statistically significant ($p<0.001$). Factors influencing capillary growth are discussed.

1. Krogh, A., J. Physiol., 1919, v52, 409.
2. Paff, G. H., Anat. Record, 1930, v46, 401.
3. Schmidt-Nielsen, K., Pennycuik, P., Am. J. Physiol., 1961, v200, 746.
4. Myers, W. M., Honig, C. R., Am. J. Physiol., 1964, v207, 653.
5. Petren, T., Sjöstrand, T., Sylven, B., Arbeitsphysiologie, 1936, v9, 376.
6. Wachtlová, M., Rakusan, K., Poupa, O., Physiol. Bohemoslov., 1965, v14, 328.

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## Effect of Hemorrhage on Hepatic Potassium Movements.* (31867)

DANIEL ANDERSEN AND WILLIAM C. SHOEMAKER
*Department of Surgical Research, Division of Surgery, Hektoen Institute for Medical Research of the Cook County Hospital, Chicago*

Early experimental studies reported potassium shifts from intracellular fluid to extracellular fluid resulting from hemorrhage and trauma(1-4). In the late stages of clinical shock, potassium shifts were suggested by increased serum potassium concentrations. However, evaluation of the significance of increased potassium levels has varied widely. According to one viewpoint, increased K may be a cause of irreversibility in shock; at the other extreme, it has been regarded as a terminal phenomenon without particular importance.

Increased potassium levels associated with increased rates of potassium release from the liver were observed in unanesthetized dogs during gradual prolonged hemorrhage(5). The possibility exists that potassium shifts may occur early after onset of hemorrhage

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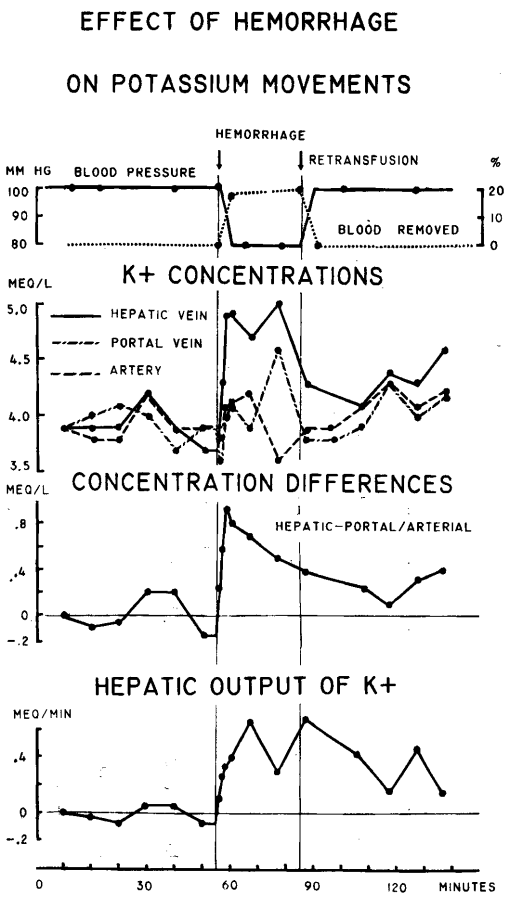


Fig. 1. Data from a representative experiment.

Upper section: Mean arterial pressure (solid line) and volume of blood removed (dotted line) during control period, after hemorrhage (indicated by first vertical line at 55 min) and after return of shed blood (indicated by second vertical line at 85 min). Removal of 20% of the blood volume resulted in an appreciable decrement of arterial pressure.

Second section: Plasma $K^+$ concentrations from arterial, portal and hepatic venous sampling sites are shown during control period, after hemorrhage and after return of shed blood. Marked increase in hepatic venous plasma $K^+$ concentration is seen almost immediately after onset of hemorrhage.

Third section: Plasma $K^+$ concentration difference across the liver is shown for the 3 periods.

Lowest section: Net rate of hepatic $K^+$ output (indicated by positive values above zero line) or uptake (indicated by values below zero line) are shown during the 3 periods. There is prompt release of K shortly after onset of hemorrhage, and delayed recovery after return of shed blood.

and may be an integral part of the response of the organism to the stress of hemorrhage. The present study was undertaken to describe the immediate potassium movements from the liver after sudden hemorrhage in intact unanesthetized dogs. Moderate-sized hemorrhage, consisting of approximately 20% of the blood volume, was used to avoid the interference of pronounced electrolyte changes, acidosis or prolonged tissue hypoxia associated with severe shock states.

*Methods and materials.* The early effects of a sudden hemorrhage on potassium shifts in the liver and the extrahepatic splanchnic area were studied in 20 experiments performed on 12 unanesthetized mongrel dogs.

1. *Experimental preparation.* Prior to the experiments, the left common hepatic vein, the portal vein and the splenic artery were catheterized with plastic tubing by a previously described technique(6). The dogs were studied 3 to 7 days after implantation of the catheters after having recovered from the operative procedure. They were fasted overnight, and studied while resting in a Pavlov stand. Blood samples were withdrawn continuously into centrifuge tubes by a constant withdrawal pump, so that contemporaneous, time-integrated samples were obtained from the three sampling sites before, during and after hemorrhage.

2. *Protocol.* Fig. 1 illustrates a representative experiment. Before the bleeding at least 6 control samples were obtained simultaneously from each of 3 sampling sites. The hemorrhage was performed by removing 20% of the calculated blood volume (17 ml/kg) from the arterial catheter during a 60 second period. At the onset of the bleeding at least 3 samples were taken from each catheter at 30 to 60 second intervals, and then 3 samples were taken over 3 minute intervals. This defined the initial 12 minute period after hemorrhage. After the first 12 minutes, the protocol was altered somewhat to answer somewhat different questions. Usually, the shed blood was returned to the dogs after 30 minutes. However, in a few experiments, bleeding was continued to the death of the dog or until sacrificed.

3. *Analytical methods.* Plasma potassium and sodium were determined by a flame photometer with internal lithium standard. Hepatic plasma flow was measured by a modified bromsulphalein method(7).

4. *Calculations.* Contemporaneous measurements of arterial, portal venous and hepatic venous concentrations of plasma potassium and sodium permit calculation of concentration differences across the liver and across the extrahepatic splanchnic area, the latter being defined as the area drained by the portal vein. The total hepatic plasma flow was measured by the bromsulphalein method. The work of Blalock and Mason (8) demonstrated that within rather narrow limits, 80% of the total hepatic blood flow entered the liver by way of the portal vein and 20% by the hepatic artery in unanesthetized dogs. The net output or uptake of electrolytes by the liver or nonhepatic splanchnic area were calculated as the product of the concentration differences and the corresponding plasma flow. Calculation of the data have shown that no important change is introduced even if the portal contribution to the hepatic flow were to vary between 60% and 100% of the total hepatic blood flow.

5. *Statistical evaluation of data.* The response to hemorrhage was evaluated by statistical analysis of the data as follows: a) The values of all experiments during all time periods in the control period were pooled and compared with similarly pooled data during the first 12 minutes after the hemorrhage. This treatment assumes that the values were time-invariant and dog-invariant. b) The means and standard errors of the means of all experiments were calculated for each sampling interval during the control period and the post hemorrhagic period. c) the values obtained from each experiment at each point in time after onset of hemorrhage were compared with the control values using the t-test for differences between correlated means.

*Results.* 1. *Plasma potassium concentration.* The data of a representative experiment are illustrated in Fig. 1, and the summary of the data of the series is shown in Table I. The data demonstrate a progressive rise in plasma K concentrations after hemorrhage in all 3 sampling sites, especially in the hepatic venous plasma. The time course of the changes in mean plasma potassium values during the first 12 minutes after hemorrhage

TABLE I. Comparison of Control Values with Each Time Period After Hemorrhage.

| | Control | Minutes after hemorrhage 0–1 | 1–2 | 2–3 | 3–6 | 6–9 | 9–12 | At maximum‡ |
|---|---|---|---|---|---|---|---|---|
| Potassium concentrations | | | | | | | | |
| Hepatic vein (mEq/l) | 3.9 ± .23 | 3.9 ± .18 | 4.0 ± .2 | 4.2 ± .23 | 4.6 ± .25 | 5.4 ± .44 | 5.9 ± .61 | 6.1 ± .59 |
| P value* | | NS† | NS | .05 | .001 | .001 | .005 | .001 |
| Portal vein (mEq/l) | 3.6 ± .14 | 3.7 ± .16 | 3.7 ± .17 | 4.0 ± .21 | 4.2 ± .25 | 4.3 ± .21 | 4.6 ± .29 | 4.7 ± .29 |
| P value | | NS | NS | .02 | .01 | .001 | .001 | .001 |
| Arterial (mEq/l) | 3.5 ± .15 | 3.5 ± .15 | 3.5 ± .14 | 3.6 ± .14 | 3.8 ± .15 | 3.9 ± .17 | 4.0 ± .20 | 4.11 ± .18 |
| P value | | NS | NS | NS | .005 | .005 | .005 | .001 |
| Hepatic plasma flow | 494 ±49 | 579 ±102 | 512 ±93 | 539 ±93 | 491 ±119 | 475 ±89 | 381 ±82 | 879 ±117 |
| (ml/min) P value | | NS | NS | NS | NS | NS | NS | .01 |
| Hepatic potassium output | .070 ± .037 | .119 ± .044 | .160 ± .060 | .120 ± .071 | .246 ± .102 | .302 ± .103 | .284 ± .073 | .499 ± .091 |
| (mEq/min) P value | | NS | NS | NS | .10 | .03 | .03 | .001 |

* P values for differences between correlated means of the control period and each sampling period.
† NS, not significant. ‡ Maximum value irrespective of the time after onset of hemorrhage.

are shown in Table I.

The mean value of the 131 control hepatic venous plasma potassium concentrations, pooled without regard to time, was 3.85 ± 0.09 (SE) mEq/l. During the post hemorrhagic period the mean of 105 hepatic venous potassium concentrations was 4.60 ± 0.15 mEq/l ($P<0.001$). The mean of 133 pooled control portal venous plasma potassium concentrations was 3.70 ± 0.05 mEq/l; after hemorrhage the mean of 105 portal venous samples was 4.09 ± 0.09 mEq/l ($P<.001$). The mean of the pooled control arterial plasma values was 3.53 ± 0.05 mEq/l; in the post hemorrhagic period the mean was 3.65 ± 0.07 mEq/l. The latter is not significantly different from control values.

When the control values of each experiment were compared with the values for each point in time after the onset of hemorrhage using the t-test for differences between correlated means, a significant increase in hepatic and portal venous potassium levels was demstrated after 2 minutes and in arterial plasma K levels after 3 minutes (Table I).

2. *Potassium concentration differences across the liver and nonhepatic splanchnic area.* In the control period there were small hepatic-portal venous and portal venous-arterial potassium concentration differences. These values increased during the 6 to 9 and 9 to 12 minute intervals after hemorrhage.

3. *Hepatic plasma flow.* The mean value for the hepatic plasma flow throughout the control periods of all experiments was 494 ± 49 (SE) ml/min. In the posthemorrhagic period the mean hepatic plasma flow was 496 ± 58 ml/min. Using the t-test for differences between correlated means, no significant difference was observed (Table I).

However, in every experiment there was an appreciable increase in hepatic plasma flow during one or more time periods after hemorrhage. The average hepatic plasma flow at the maximum value was 879 ± 117; this was significantly different from the control values ($P<0.01$).

4. *Hepatic potassium output.* The rates of hepatic potassium output in the control period and after the hemorrhage are illustrated in Fig. 1. The increased hepatic outputs of potassium are significantly different from the control values in the 6-12 minute periods (Table I).

When all the values of all experiments are taken together, the mean rate of hepatic potassium output in the control period was 0.07 ± 0.04 mEq/min. After hemorrhage the mean rate of potassium output was 0.24 ± 0.07 mEq/min ($P<2.5\%$). After hemorrhage the average hepatic potassium output taken at the maximal rate was 0.50 ± 0.09 mEq/min ($P<0.001$).

5. *Extrahepatic splanchnic potassium output.* The mean output of potassium from the extrahepatic splanchnic area in the control period was 0.08 ± 0.03 mEq/min. After hemorrhage, the mean of all potassium outputs pooled without respect to time was 0.11 ± 0.05 mEq/min. After hemorrhage the average potassium output taken at maximal values was 0.25 ± 0.05 mEq/min.

6. *Plasma sodium concentrations.* The means of the pooled values for hepatic venous, portal venous and arterial plasma Na concentrations in the control period were 144.3 ± 0.5, 144.6 ± 0.5 and 142.8 ± 0.4 mEq/l, respectively. After hemorrhage these values, in the same order, were 144.6 ± 0.1, 144.8 ± 0.5 and 143.2 ± 0.6 mEq/l. There were no significant differences between correlated means or between the pooled values.

7. *Hepatic sodium output.* In the control period the mean rate of hepatic Na output was 0.11 ± 0.22 mEq/min. After hemorrhage this value was 0.19 ± 0.31 mEq/min. The average minimal hepatic sodium output obtained in each experiment after hemorrhage was—1.99 ± 0.64 mEq/min; *i.e.*, an uptake of 1.99 mEq/min. When compared with corresponding control values, a significant difference was found ($P<0.01$). This suggests a transient hepatic uptake of Na during the post hemorrhagic period.

*Discussion.* The total output of potassium from the liver during the first 12 minute period after hemorrhage was calculated from the area under the time-rate curve. The net increase over control for the series was 2 mEq. If this amount of potassium was distributed evenly in the extracellular fluid com-

partment, it would increase the potassium concentration in arterial plasma about 0.45 mEq/l. The actual increase in arterial potassium concentration averaged 0.5 mEq/l. It follows that the potassium released from the liver accounts for almost all of the potassium increase in the extracellular fluid compartment. Probably the extrahepatic splanchnic tissues also contribute some potassium to this compartment. Other tissues probably contribute little or nothing. We can conclude that the potassium loss in the early stages of sudden acute hemorrhagic hypovolemia is not a universal reaction of the body's cells, but is specific for certain tissues or organs, the liver being the most important of these.

The potassium loss occurs within a few minutes after onset of hemorrhage, and measurements of hepatic plasma flow suggest a short increase about the same time. This is similar to the pattern found after epinephrine stimulation(9-12). Also it is known that increased catecholamine concentrations were found immediately after the onset of a hemorrhage(13). Therefore, it is reasonable to relate the potassium loss from the liver as found in this study to epinephrine action.

*Summary.* The immediate effect of a sudden, acute, moderate-sized hemorrhage on hepatic and extrahepatic splanchnic movements of potassium and sodium has been studied in the unanesthetized dog. The net rates of hepatic and extrahepatic splanchnic uptake or output of potassium and sodium were calculated from arterial, portal and hepatic venous plasma concentrations and hepatic plasma flow measurements. An increase in plasma potassium concentration was established in the hepatic vein and portal vein 2 minutes and in the splenic artery 3 minutes after the hemorrhage. A significant increase in net hepatic potassium output was found in the post hemorrhagic period after 3-6 minutes, and a transient increase in the output of potassium from the extrahepatic splanchnic area was suggested. No consistent changes were found in the plasma concentrations of sodium after hemorrhage, but a transient hepatic uptake of sodium in the post-hemorrhagic period was suggested. Likewise a transient increase was found in the hepatic plasma flow after hemorrhage. These changes found after hemorrhage are similar to changes found after an injection of epinephrine. This suggests that the effect of an acute hemorrhage on hepatic potassium movements may result from epinephrine action.

1. Thaler, J. I., Proc. Soc. Exp. Biol. and Med., 1935, v33, 368.

2. Zwemer, R. L., Scudder, J., Surg., 1938, v4, 510.

3. Brues, A. M., Cohn, W. E., Kety, S. S., Nathanson, I. T., Nutt, A. L., Tibbetts, D. M., Zamecnik, P. C., Aub, J. C., J. Clin. Invest., 1945, v24, 835.

4. Root, W. S., Allison, J. B., Cole, W. H., Holmes, J. H., Walcott, W. W., Gregersen, M. I., Am. J. Physiol., 1947, v149, 52.

5. Shoemaker, W. C., Walker, W. F., Turk, L. N., Surg. Gyn. Obst., 1961, v112, 327.

6. Shoemaker, W. C., Walker, W. F., Van Itallie, T. B., Moore, F. D., Am. J. Physiol., 1959, v196, 311.

7. Shoemaker, W. C., J. Appl. Physiol., 1960, v15, 473.

8. Blalock, A., Mason, M. F., Am. J. Physiol., 1936, v117, 328.

9. D'Silva, J. L., J. Physiol., 1934, v82, 393.

10. LeVeen, H. H., Falk, G., Surg. Forum, 1961, v12, 345.

11. Shoemaker, W. C., Finder, A. G., Proc. Soc. Exp. Biol. and Med., 1961, v108, 248.

12. Shoemaker, W. C., Turk, L. N., Moore, F. D., Am. J. Physiol., 1961, v201, 58.

13. Walker, W. F., Zileli, M. S., Reutter, F. W., Shoemaker, W. C., Friend, D., Moore, F. D., ibid., 1959, v197, 773.

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