BIOCHEMICAL STUDIES ON SHOCK

II. The Rôle of the Peripheral Tissues in the Metabolism of Protein and Carbohydrate during Hemorrhagic Shock in the Rat [*]

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In a previous report (1) it was shown that during hemorrhagic shock in the rat there is a progressive rise in the blood levels of amino nitrogen, keto acids as pyruvate, and lactate. The blood sugar falls, provided epinephrine hyperglycemia is prevented either by previous suprarenodemedullation or by reduction of the liver glycogen level by fasting. These changes were interpreted as being due in part to the effects of peripheral circulatory failure on the hepatic circulation, resulting in anoxia to that organ, and in part to the effects of decreased circulation to the peripheral tissues on the metabolism of protein and carbohydrate by those tissues. Since, however, both factors undoubtedly participate to produce the changes observed in the blood, the experiments reported here were designed to analyze these changes further in terms of the relative contributions of the liver and the peripheral tissues to the total metabolic picture. By eliminating the liver surgically and then reducing the circulation by hemorrhage, it becomes possible to establish the contributions of the peripheral tissues to the biochemical changes. For this purpose rats were eviscerated, the entire gastrointestinal tract from esophagus to rectum, the spleen and the pancreas being removed, and the circulation to the liver being excluded. When shock is then induced by hemorrhage in such a preparation, any blood changes other than those produced by evisceration alone can be attributed to the effects of peripheral circulatory failure on the metabolism of the remaining tissue.

It was shown by this method that an increased rate of protein breakdown by the peripheral tissues accounts for a considerable proportion but probably not all of the observed blood amino nitrogen rise during shock, while the blood sugar, lactate, and pyruvate changes are largely determined by alterations in the metabolism of the peripheral tissues.

Methods

The methods used were the same as those described in the previous report (1, 2) with the exception that the keto acids were determined on heparinized blood, im-

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mediately precipitated by 10 per cent trichloracetic acid. Evisceration was performed by the technique used elsewhere by one of us (3). Under nembutal anesthesia a midline incision was made and double ligatures passed about the rectum, the inferior and superior mesenteric arteries, the celiac axis, and the portal vein. The rectal and arterial ligatures were tied first and then the portal, to avoid back-flow of blood into the intestines, and the entire gastrointestinal tract from rectum to esophagus, the pancreas, and spleen were removed. The esophagus was left open to permit swallowing. The operation can be performed in about 5 minutes, is associated with negligible blood loss, and postoperatively the animals show no obvious signs of shock. In the studies on blood amino nitrogen levels, rats fasted 24 hours were eviscerated 1 hour before they were subjected to a hemorrhage equivalent to 2 per cent of their body weight. For the pyruvate and lactate experiments, suprarenodemedullated rats were employed to avoid the effects of epinephrine discharge on the blood levels of these substances. Further, these animals were kept under sodium pentobarbital anesthesia for 1 hour before operation and hemorrhage was begun immediately after evisceration since these animals are known to be more sensitive to evisceration than are animals with intact suprarenal medullae.

RESULTS

The blood amino nitrogen and sugar levels were studied in a control series of nine fasted eviscerated rats and in seven fasted eviscerated rats from which blood equivalent in amount to 2 per cent of the body weight was removed 1 hour after evisceration. Fig. 1 illustrates the effects of these procedures on the blood amino nitrogen levels. It will be noted that the bled rats survived approximately $2\frac{1}{2}$ hours while the control eviscerated rats survived about 5 hours. All rats died with convulsions due to hypoglycemia. In the control animals there was a progressive increase in blood amino nitrogen content amounting to 28 mg. per cent in 5 hours. In the bled rats the rate of rise in amino nitrogen was identical with that in the controls during the 1st hour, but once bleeding was begun the rate of accumulation of amino nitrogen was considerably enhanced. Thus at the time of death $2\frac{1}{2}$ hours after bleeding the blood amino nitrogen had risen over 18 mg. per cent, an increase not achieved in the control rats until over 4 hours had elapsed. Fig. 2 shows the changes in the blood sugar levels in the same animals. While in the control animals there was a slow fall in blood sugar, hemorrhage resulted in a rapid and steady decline in blood sugar until the animals died in hypoglycemic convulsions. Indeed, it would seem that hypoglycemia was one of the limiting factors in the survival of these rats.

Since epinephrine discharge during evisceration or in hemorrhage may cause large and irregular increases in the blood sugar, lactate, and pyruvate, the blood levels of these substances were studied in rats which had been previously suprarenodemedullated. Hemorrhage was begun in these animals immediately after evisceration; blood equal in amount to $1\frac{1}{2}$ per cent of the total body weight was removed in each case. Fig. 3 demonstrates the rate of fall in blood sugar in the bled and control suprarenodemedullated-eviscerated rats. Again the

very much more rapid rate of disappearance of glucose from the blood is seen in the bled rats. In Fig. 4 are recorded the effects of evisceration and of
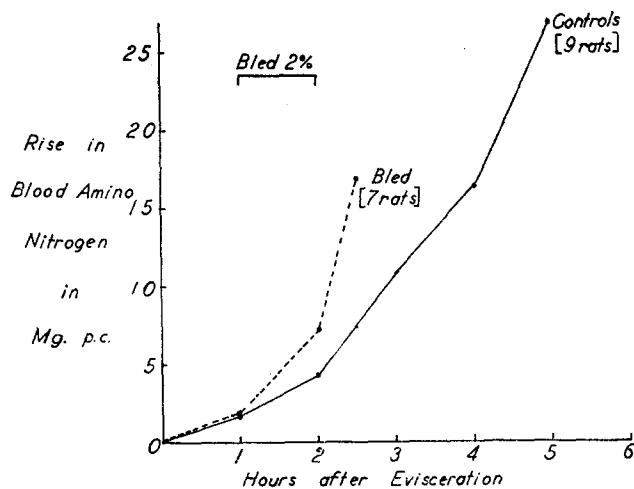

FIG. 1. The effect of hemorrhage on the blood amino nitrogen content of eviscerate (functionally hepatectomized) rats. The control rats were eviscerated and the blood amino nitrogen followed until death. The bled rats were subjected to a hemorrhage equivalent to 2 per cent of their body weight between the 1st and 2nd hours after evisceration.
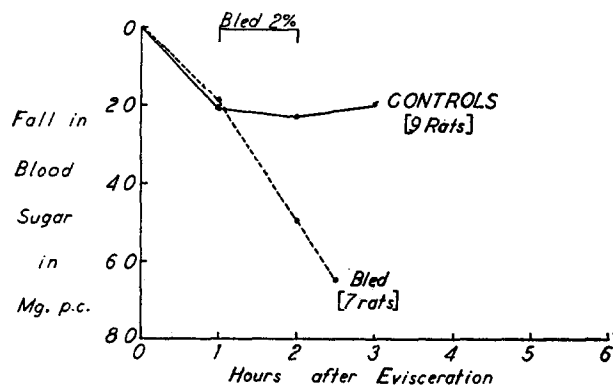

FIG. 2. The effect of hemorrhage on the blood sugar levels of eviscerate rats. The rats were treated as in Fig. 1.

evisceration with hemorrhage on the blood pyruvate and lactate levels. In both series death was associated with convulsions, so that any interpretation of the changes in the terminal specimens must be made with this factor in mind. Following evisceration, there was a gradual increase in the blood pyruvate in the control rats, with a parallel rise in lactate; the ratio of lactate
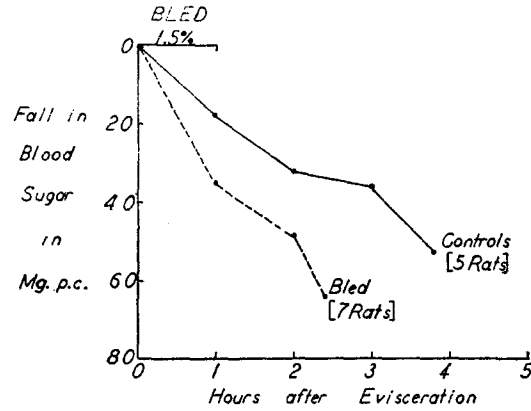
FIG. 3. The effect of hemorrhage on the blood sugar levels of eviscerate supra-renodemedullated rats. The bled rats were subjected to a hemorrhage equivalent to 1.5 per cent of their body weight during the 1st hour after evisceration.
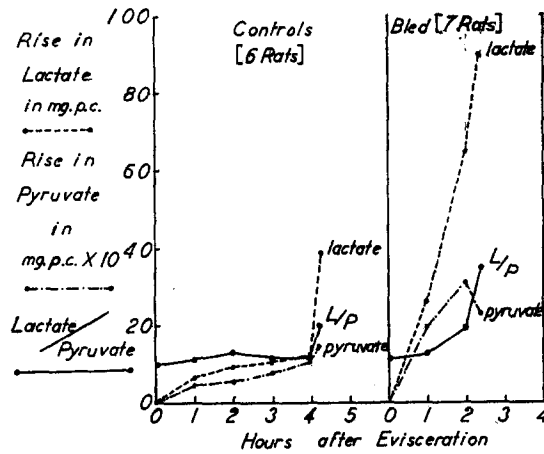

FIG. 4. The effect of evisceration and evisceration plus hemorrhage on the blood lactate and pyruvate levels of suprarenodemedullated rats. The control rats were eviscerated and the blood lactate and pyruvate followed until death. The latter was associated with convulsions in all cases, which accounts for the sudden sharp rise in lactate and pyruvate terminally. The bled rats were subjected to a hemorrhage equivalent to 1.5 per cent of their body weight immediately after evisceration. Convulsions preceded death. The rats were kept under nembutal anesthesia for 1 hour before evisceration.

to pyruvate remained constant until the terminal specimens taken during convulsions, when the lactate had risen more sharply than the pyruvate. In

contrast to this was the very rapid rise in pyruvate and lactate in the bled rats, with the lactate rise outstripping the pyruvate and resulting in a mounting lactate to pyruvate ratio. Of interest was the terminal pyruvate fall at the time of the convulsions. In the control eviscerate rats the increases in blood lactate and pyruvate were small and did not approach those seen either in the intact rat subjected to hemorrhage (1), or in the bled eviscerate rat.

DISCUSSION

In the rat suffering from peripheral circulatory failure, a progressive increase in the blood amino nitrogen content is a characteristic feature. Although the method of analysis (2) is not entirely specific for amino acid nitrogen, 28 per cent of the uric acid nitrogen also being determined, all but a very small fraction of the amino nitrogen found in blood must be amino acid nitrogen. As has been pointed out elsewhere (1), appreciable and persistent elevations in blood amino acids are not seen, even after injections of relatively large amounts of these substances, unless there is impairment of liver function. Any elevation in the amino acids of the blood will depend in part on the degree of hepatic disability present and in part on the rate of amino acid production from tissue proteins or food. The rise in blood amino nitrogen in the bled rat (1) is in many cases equal to or greater than that which occurs in the liverless rat. Since in the latter case deamination is already reduced to a minimum, a greater increase in blood amino nitrogen in the shocked animals would have to be due to a greater rate of amino nitrogen production during shock. In the experiments described in this report, direct measure of this factor was obtained by comparing the rate of rise in blood amino nitrogen in the liverless rat with that in the liverless rat subjected to hemorrhage and shock. The more rapid accumulation of amino nitrogen in the blood of the bled liverless rats thus indicates an increased rate of protein breakdown in the peripheral tissues, since the viscera have been removed. Within the periods of observation in these experiments, nephrectomy does not influence the blood levels of amino acids. Amino acid excretion or deamination by the kidneys would not seem to be significant factors in the differences observed in the shocked animals. Comparison of the two curves in Fig. 1 reveals a much greater accumulation of amino nitrogen in the blood of the bled rats than in the controls in the hour and a half from the beginning of the hemorrhage, representing a considerably more rapid rate of protein breakdown in the shocked rat.

The rising blood amino nitrogen concentration during hemorrhagic shock in the otherwise normal rat may be attributed partly to increased protein degradation in the peripheral tissues and partly to failure of the liver to assimilate the amino acids resulting from this breakdown, because of the decreased blood flow to and anoxia of the liver (4). This degree of hepatic failure makes it possible to detect by study of the blood amino nitrogen level an increase in

protein catabolism which might otherwise be missed if the liver maintained its normal ability to handle large amounts of amino acids.

An increase in protein catabolism after hemorrhage has previously been demonstrated by several investigators (5, 6) who studied the urinary nitrogen excretion. Similarly after trauma and burns (7) an increased nitrogen excretion has been observed. In the case of burns Glenn *et al.* (8) have recently reported an increase in blood and lymph amino acid nitrogen. Our results show that the generalized tissue anoxia resulting from hemorrhage produced a rapid breakdown of peripheral tissue protein just as do burns, trauma, or local anoxia by tourniquet In the latter cases it is probable that the increased protein catabolism is not exclusively in the traumatized tissue, but also occurs generally whenever the circulation is sufficiently depressed.

A comparison of the blood changes in sugar, lactate, and pyruvate in the eviscerate rat and the eviscerate shocked rat indicates that these changes are primarily conditioned by the state of the peripheral tissues. In the liverless preparation there is a progressive fall in the blood sugar as this substance is utilized and, as no new source is available, the animal eventually dies in hypoglycemic convulsions. The lactate and pyruvate levels slowly rise, but maintain a constant ratio to each other, except in the terminal specimen which is influenced by the effects of the convulsion. The gradual rise in these substances is probably due in part to the absence of the liver which would normally utilize lactate and pyruvate. The persistence of a normal lactate/pyruvate ratio until terminally suggests that carbohydrate is being normally metabolized by the eviscerate preparation. By comparison, in the bled eviscerate rat, glucose disappears at a much more rapid rate and lactate and pyruvate accumulate rapidly; the lactate increases in the blood faster than sugar disappears, and there is a rising lactate/pyruvate ratio. These facts suggest an increasing predominance of anaerobic over aerobic metabolism of carbohydrate in muscle (9). The more rapid disappearance of glucose in the shocked preparation may be a manifestation of the lesser efficiency in terms of energy yield of the anaerobic metabolism of carbohydrate. Since the rates of change in blood lactate and pyruvate are similar in the intact bled and the eviscerate shocked rats but are much greater than those seen in the control eviscerate rats, hepatic failure alone would not seem to be a significant factor in producing these changes during shock. On the contrary, peripheral anoxia would seem to be largely responsible.

SUMMARY

The changes in the blood levels of amino nitrogen, glucose, lactate, and pyruvate were compared in eviscerate (liverless) rats and eviscerate rats subjected to hemorrhage, in order to establish the rôle of the peripheral tissues in the blood changes during shock. It was found that:—

1. The blood amino acids accumulate at a more rapid rate in the bled liverless rats than in the control liverless animals.

2. The blood sugar falls more rapidly in the liverless rat after hemorrhage, both in animals with intact suprarenal glands and those with enucleated suprarenal medullae.

3. The blood lactate and pyruvate rise slowly in the liverless rat, but maintain a constant relation to each other except terminally when convulsions occur. In the bled liverless rat both lactate and pyruvate increase much more rapidly than in the control liverless rat, and the lactate/pyruvate ratio also increases.

These data are interpreted to indicate that a decrease in liver function during hemorrhagic shock serves to make apparent a considerable increase in peripheral protein catabolism and accentuates the effects of an increased carbohydrate utilization by the periphery. The lactate and pyruvate changes are determined chiefly by anoxia of the peripheral tissue and probably indicate an increasing predominance of anaerobic over aerobic metabolism of carbohydrate in muscle. The liver plays a negligible rôle in the lactate and pyruvate changes in shock.

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