BIOCHEMICAL STUDIES ON SHOCK

I. The Metabolism of Amino Acids and Carbohydrate during Hemorrhagic Shock in the Rat*

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The problem of shock has been the subject of intensive investigation for many years by physiologists, surgeons, and clinicians. While important advances have been made in our understanding of the cardiovascular dynamics of shock, the rôle of fluid loss, and the importance of the colloidal osmotic pressure of the proteins of the blood stream, no systematic studies of the metabolic aspects of shock have been carried out, except those having a bearing on the plasma proteins and electrolytes.

Implicit in the syndrome known as shock or peripheral circulatory failure, regardless of initiating factors, and even in its earliest stages is a decrease in blood flow and hence oxygen supply to certain tissues. The maintenance of an adequate oxygen supply to vital organ systems is essential to life. Indeed, the earliest circulatory response of the organism in shock, *i.e.*, peripheral vasoconstriction, has been interpreted as an attempt by the body to maintain the circulation of the most sensitive tissues, such as the central nervous system, at the expense, if necessary, of less vulnerable tissues. However, no tissue is entirely immune to the effects of oxygen deprivation for any length of time and in order to function at an oxygen tension lower than normal, certain more or less profound changes in the metabolic pattern of the tissue must take place. Moreover, as is well known, the efficiency, in terms of energy yield, of reactions taking place at lowered oxygen tension may be considerably less than normal so that to maintain function a greater supply of nutritive substrate and possibly of certain respiratory enzymes may be necessary. Just as certain reflex compensatory changes take place in circulation, so too, metabolic responses may follow in order to meet new needs.

In this and subsequent studies to be reported, an attempt was made to establish a metabolic pattern during various phases of shock. Because of its

uniformity and because more is known of the details of metabolism of the rat, this species has been used exclusively in these experiments.

Methods and Materials

Male albino rats of the Sprague-Dawley strain, weighing from 200 to 250 gm., were used throughout these experiments. The animals were fed a diet of purina dog chow and in all cases were fasted for 24 hours before being studied. In those animals in which suprarenalectomies were done, the operation was generally performed 24 to 48 hours before the experiment and the animals were maintained on pellets of desoxycorticosterone acetate implanted subcutaneously. No sodium chloride was added to the drinking water and the diet was the same as for the intact rats. Supra-reno-demedullations were carried out on young rats weighing 100 to 150 gm. and 3 to 5 weeks were allowed to elapse for full regeneration of the suprarenal cortices. All experiments were performed under anesthesia with sodium pentobarbital (nembutal) administered intraperitoneally, in a dose of 4 mg. per 100 gm. for intact rats and 3 mg. per 100 gm. for suprarenalectomized animals. Subsequent doses were given as needed. However, it was found that once shock had been established no further anesthesia was necessary.

Shock was induced by bleeding from the cut tail. In one group the animals were bled slowly over a period of 2 to 5 hours until obvious signs of profound shock were present, and it became difficult or impossible to obtain more blood from the tail. This usually occurred when an amount of blood equivalent to 3.0 to 4.2 per cent of the body weight had been removed from intact rats and from 2.0 to 3.0 per cent in suprarenalectomized rats. In the second group, blood equal to 2.5 to 3.2 per cent of the body weight of normal rats was removed in 1 hour and the animals were then studied until they died, or for 24 hours if they survived; 2.0 to 2.5 per cent was removed in the suprarenalectomized animals. Those rats which survived were allowed free access to water after the anesthesia wore off but were given no food. Animals dying in shock, as well as those sacrificed at 24 hours, were autopsied, partic-ular attention being paid to the suprarenal glands, which will be the subject of an-other report. The survival rate after hemorrhage equivalent to 2.5 to 3.2 per cent of the body weight was found to vary considerably with atmospheric conditions, being very low during hot humid weather, and to vary slightly with different batches of animals. Attempts to produce a "standard" shock preparation based on the removal of a given amount of blood in 1 hour proved unsuccessful.

The criteria for shock were primarily clinical, *i.e.*, pallor, cyanosis, cold extremities, sluggish or absent blood flow from the cut tail, tachypnea, and the failure to require further sodium pentobarbital to maintain anesthesia. Blood pressures were de-termined in several instances by direct cannulation of the carotid artery, heparin being used as an anticoagulant. The pressure was read on a mercury manometer. Heparin in saline was used in the system.

Blood sugar was determined by the Somogyi micromethod (1); liver glycogen, by the method of Good, Kramer, and Somogyi (2). Keto acids, as pyruvic acid, were determined by a modification of the method of Bueding and Wortis (3) using 0.2 cc. of blood. To avoid the variations in blood keto acid levels due to muscular move-ment and to the effect of epinephrine discharge, all animals on which keto acid de-

terminations were performed were kept under anesthesia for at least 1 hour before the first blood sample was taken. The blood pyruvate level of normal fasted rats under these circumstances was found to vary between 0.60 and 1.45 mg. per cent, with a mean of 0.98 mg. per cent. Blood amino acid nitrogen was determined by Frame's micromodification of Folin's colorimetric method, using sodium β-naphthoquinone sulfonate (4); lactic acid, by the method of Barker and Summerson (5). Blood cell volume was measured by the method of Meyerstein (6) on heparinized blood. Unless otherwise indicated, all determinations were done on whole blood.

RESULTS

In the normal fasted rat subjected to slow hemorrhage amounting to 3.5 to 4.2 per cent of the body weight over the course of 4 to 5 hours, the blood levels of amino acid nitrogen, keto acids, as pyruvate, and glucose, followed a characteristic and reproducible pattern. Fig. 1 illustrates these changes in a group of 8 rats which survived 4 to 5 hours. It will be noted that the earliest change is a rise in the blood keto acids, the level of which mounts rapidly and progressively during the course of the hemorrhage. The blood sugar in this particular preparation shows little tendency to rise and during the latter part of the experiment falls. In some experiments there was terminally a marked fall to hypoglycemic levels and animals dying in shock occasionally exhibited convulsive movements. The failure of the well known hyperglycemia of shock to occur in these experiments is probably attributable on the one hand to the fact that the liver glycogen of 24-hour fasted rats that had been receiving a chow diet (68 per cent carbohydrate, 19 per cent protein) is extremely low, and on the other hand to the fact that during continuous bleeding any sugar derived from increased glycogenolysis in the liver is promptly utilized. Fasted rats raised on a high protein diet (ground beef) and non-fasted chow-fed rats demonstrated the characteristic hyperglycemia when shocked in the same manner as the above group of animals. The former animals had liver glycogen levels of 1.15 gm. per cent (7) at the end of a 24 hour fast and from a metabolic standpoint are similar to dogs which are customarily fed high protein diets. As will be shown later, chow-fed rats subjected to a single and less severe hemorrhage from which they survive do show a hyperglycemia, demonstrating that even with low liver glycogen levels glycogenolysis takes place, but that the blood sugar rise may be masked by the immediate utilization of the liberated glucose (cf. Fig. 1). In a few experiments, lactic acid determinations were done. A slight rise in this constituent was observed early during hemorrhage and a striking elevation during the latter part of the experiment, reaching 70 mg. per cent or higher. The amino acid nitrogen characteristically showed a slight decrease at first followed by a rapid rise. The nature of the blood amino acid nitrogen change will be considered in greater detail later.

Since discharge of epinephrine is a well known accompaniment of shock, the above experiments were repeated on suprareno-demedullated rats to determine

whether any of the above changes could be attributed to the effects of this hormone. In the normal rat, the injection of epinephrine is followed by a rise in the blood sugar and blood keto acids (8) and a slight fall in blood amino acid nitrogen (8). Fig. 2 illustrates the effect of slow hemorrhage in a suprareno-demedullated rat. It will be noted that the blood changes are in the same direction as those in the normal rat with the exception that in no case was there any tendency for the blood sugar to rise; rather, there was a progressive decline
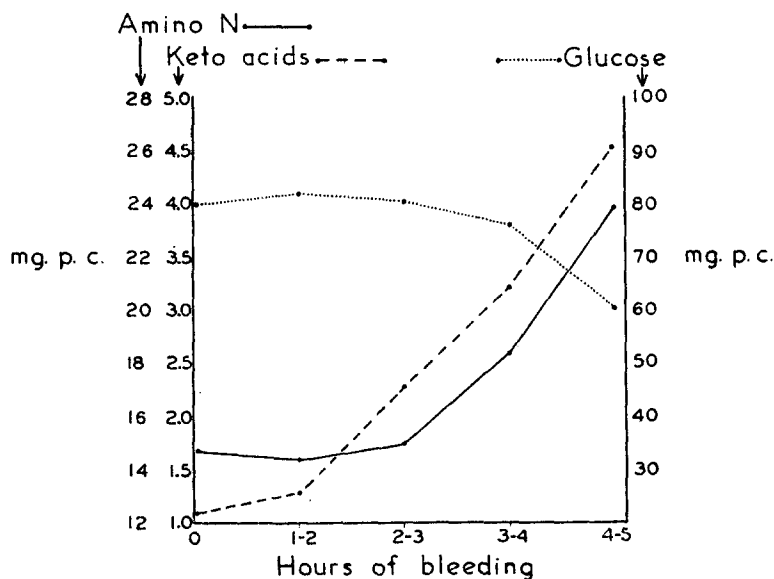

FIG. 1. The changes in the blood amino acid nitrogen, keto acids, as pyruvate, and glucose in 8 normal rats during hemorrhage. The rats were bled slowly, an amount equivalent to 3.0 to 4.2 per cent of the body weight, until clinical evidences of shock appeared and they died in 4 to 5 hours.

from the beginning of hemorrhage. The same changes were observed in fed suprareno-demedullated rats which presumably had normal liver glycogen levels. The keto acid rise occurred just as in the normal rat, indicating that epinephrine discharge alone is not responsible for this change in normal animals. And, finally, the amino acid nitrogen rise was not influenced by suprareno-demedullation.

During recent years there has been much interest in the rôle of the suprarenal cortex in shock. Suprarenalectomized animals are notoriously sensitive to shock-inducing procedures. Morphological changes have been described in the suprarenal cortices of shocked animals (9). In rats shocked by hemorrhage, as described above, lipoid depletion of suprarenal cortical cells has been noted

as early as 1 hour after hemorrhage, and measurable hypertrophy of the supra-renal cortex in 24 hours (10). In view of this and of the known rôle of the suprarenal cortex in carbohydrate and protein metabolism, it was of interest, therefore, to determine the effect of suprarenalectomy on the blood changes in shock. Fig. 2 demonstrates that, except for the immediate downward trend of the blood sugar, the changes are the same as in the normal rat. However, at this time it cannot be stated whether the changes in the amino acid nitrogen and keto acids occur to the same degree as in the normal animal. Both the suprareno-demedullated and suprarenalectomized preparations are more sensitive to hemorrhage than the normal rat, the former succumbing when 2.0 to
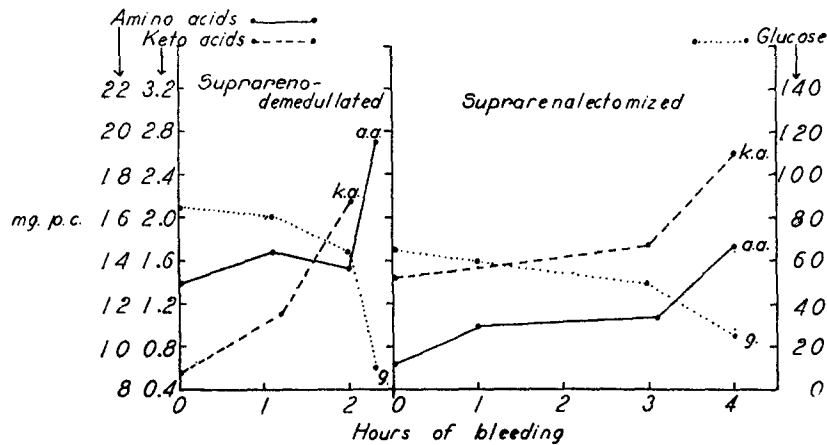

Fig. 2. The changes in the blood amino acid nitrogen, keto acids, as pyruvate, and glucose in suprareno-demedullated and suprarenalectomized rats during hemorrhagic shock.

2.5 per cent of the body weight, as blood, had been removed. It is thus difficult to compare them with the normal rat without having more exact data on the status of the circulatory system. The greater tendency to hypo-glycemia in the suprareno-demedullated and suprarenalectomized rats may be one limiting factor in both the survival and the degree of change in keto and amino acids.

In the experiments just described, the hemorrhage always resulted in a fatal outcome so that no relationships could be established between the severity of the shock and the changes in the blood. For this reason a different method of bleeding was adopted. The rats were bled approximately 3 per cent of the body weight in an hour and then followed at intervals thereafter until they died, or for 24 hours if they survived.

In Fig. 3 are recorded the blood keto acid levels in 10 rats, 5 of which died in

4 hours and 5 survived for 24 hours. Those that died showed the character-
istic steep rise in the blood pyruvate. In the surviving group the rate of rise
in keto acids was much less, a peak being reached in 4 hours and in 5 hours the
level had almost returned to normal.

Under most circumstances in which it has been studied previously, the level
of the blood amino acids has been found to be quite constant. Consistent
elevations are rarely observed except when liver function is impaired or after
hepatectomy. Thus, the finding of an elevated amino acid nitrogen level
during shock has been subjected to more detailed analysis. Fig. 4 illustrates
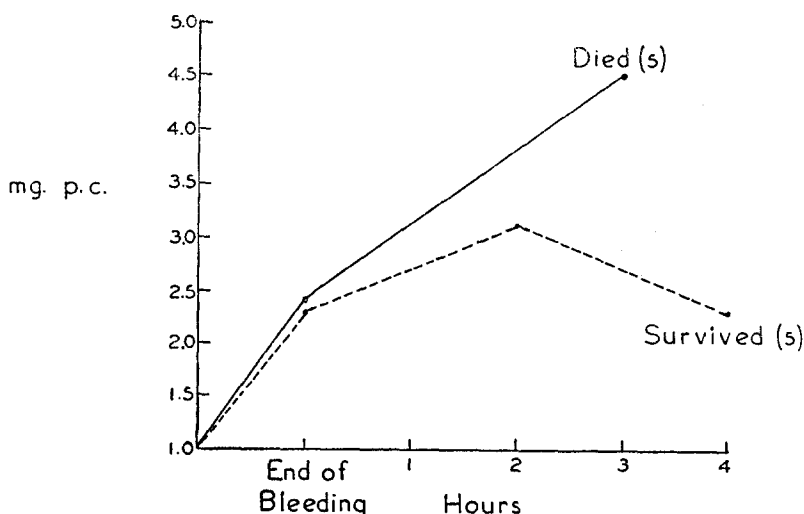the amino acid nitrogen changes in a group of 31 rats of which 14 survived and

FIG. 3. The changes in the blood keto acids as pyruvate following hemorrhage,
equivalent to 3.0 per cent of the body weight in normal rats.

17 died at varying periods after having been bled in the manner just described.
It will be noted that in those animals which survive there is an initial fall in
blood amino acid nitrogen at the end of bleeding followed by a slow rise which,
however, never exceeds the initial value by more than 2 to 3 mg. per cent.
24 hours after the hemorrhage, the whole blood amino acid nitrogen was found
to be significantly below normal. However, as will be shown below, this fall
is due largely to the decreased proportion of erythrocytes in the blood at this
time. In those animals that die in shock, the rate of rise in amino acid nitrogen
was found to be proportional to the survival period. If the rats survived more
than 4 hours, an initial decrease in amino acids was noted. The eventual height
which the blood levels attained was greater the longer the animal remained in
shock. The amino acid nitrogen pattern described in Fig. 4 has been repeat-
edly confirmed in a large series of animals. In no case has an animal died

after a hemorrhage without an elevation of the blood amino acid nitrogen level. Moreover, in the untreated animal, the change in the whole blood amino acid level at any given time after a hemorrhage has been a prognostic index which has proven to be of considerable value, particularly in judging the condition of an animal which was to be sacrificed for tissue studies during shock.

Since it is known that the amino acids are unevenly distributed between cells and plasma, the former having 2 to 3 times as much as the latter in the rat (11) and since during the course of and after hemorrhage there is a progressive decrease in the red cell volume, which amounted in our experiments to a fall in the hematocrit from 47 per cent to 40 per cent at the end of bleeding,
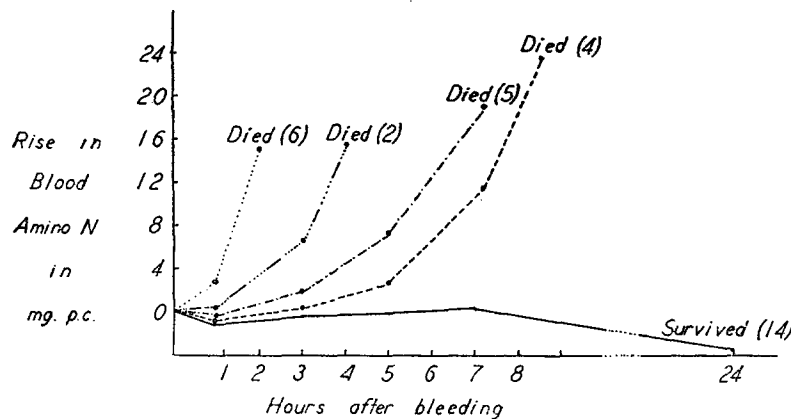

FIG. 4. The effect of blood loss equivalent to 3 per cent of the body weight in 1 hour on the blood levels of amino acid nitrogen in normal rats.

to 32 per cent 4 hours after bleeding, to 29 per cent 6 hours and 21 per cent at 24 hours, the process of hemodilution alone should result in a decrease in the whole blood amino acids. For that reason a study was made of the whole blood and plasma amino acid levels during shock (Fig. 5). Here it is clearly evident that in the group surviving the hemorrhage the plasma amino acid levels do rise, but the hemodilution obscures this change in the whole blood. In the animals that die, the plasma amino acids rise at a more rapid rate than do those in the whole blood. The fact that the hemodilution following hemorrhage itself causes a decrease in the whole blood amino acid levels lends added significance to any rises that may occur during hemorrhagic shock.

In all the experiments so far described, the criteria for shock were entirely clinical and no correlation was made between chemical and hemodynamic findings. Therefore, it became of importance to attempt to determine what time relationship may exist between the rise in blood amino acid nitrogen and the level of the blood pressure. Blood pressures were measured by direct

cannulation of the carotid artery in a series of 11 rats, a representative example of which is found in Fig. 6. Here it is seen that the blood amino acids begin to rise only when the mean effective blood pressure has fallen to between 80 to 90 mm. of mercury and that the rise thereafter was progressive even though in this case all the blood removed was replaced and the hematocrit was elevated above the initial value. In Fig. 7 is plotted the change in blood amino acid nitrogen from a number of determinations in 11 rats against the blood pressure. Each point on this curve represents three to eleven amino acid nitrogen determinations at a given blood pressure. The sharp break that
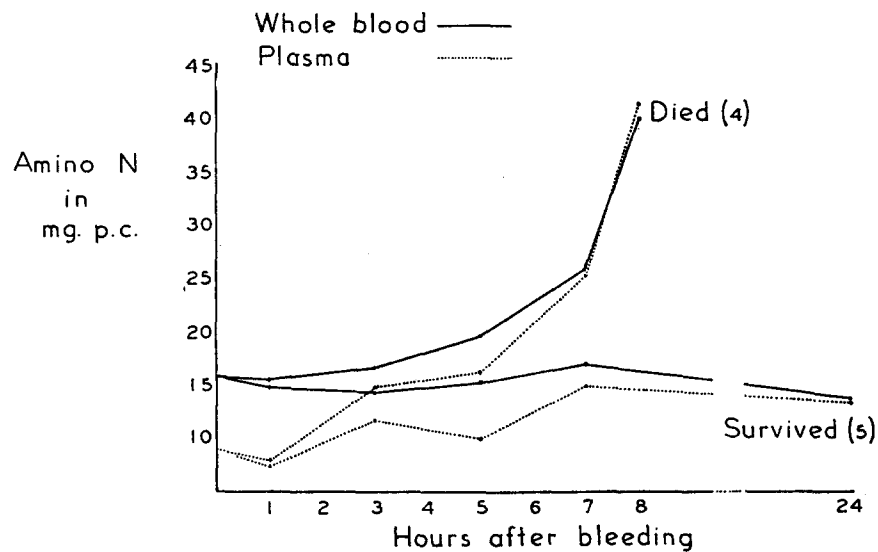

FIG. 5. Plasma and whole blood amino acid nitrogen levels in normal rats after hemorrhage.

occurs in this curve between 80 to 90 mm. Hg is particularly noteworthy and suggests that the amino acid elevation is secondary to changes in the circulation. Evidence will be presented later suggesting that this critical break is dependent on changes in hepatic circulation.

In the early part of this paper data were presented suggesting that the epinephrine hyperglycemia commonly reported during shock may mask certain more fundamental changes. The blood sugar of the shocked suprareno-demedullated rat has therefore been studied further (Fig. 8). In the intact rat (curve E) hemorrhage for 1 hour produced a sustained hyperglycemia if the animal survived. This was in contrast to the effect of continuous and fatal hemorrhage in an otherwise similar rat (Fig. 1) in which the blood sugar showed little tendency to rise. In the demedullated rat, however, the picture was different. First of all, sodium pentobarbital itself will cause some decrease
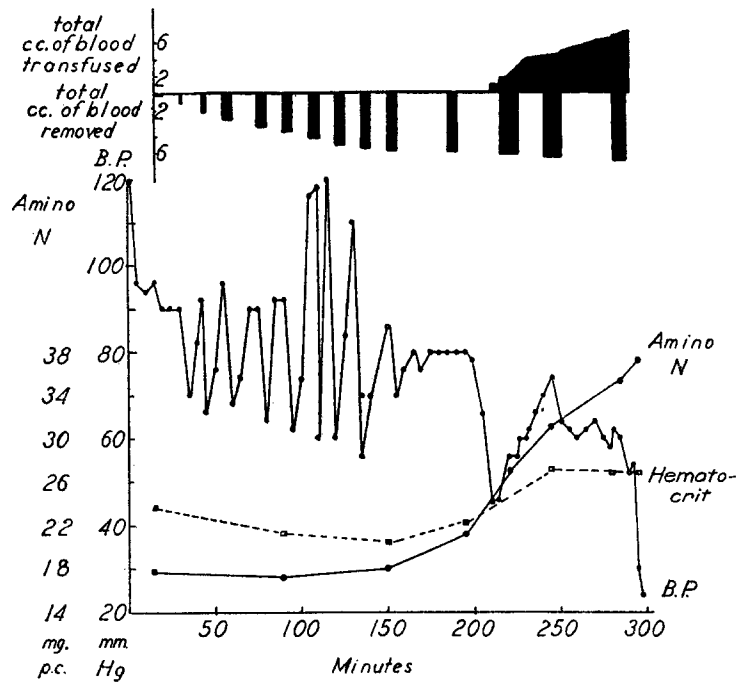
FIG. 6. The blood pressure, hematocrit, and blood amino acid nitrogen during hemorrhage in an intact rat. When the blood pressure had fallen to 48 mm. of mercury, all the blood removed was replaced during the subsequent hour and a half.
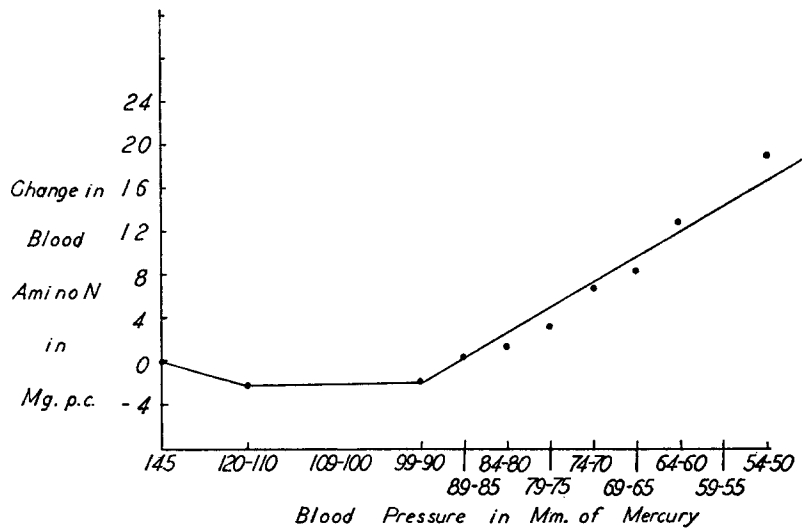

FIG. 7. The relation of the arterial blood pressure to the blood amino acid nitrogen levels in 11 rats during hemorrhage.

in blood sugar in the demedullated rat (curve A). However, the immediate fall in blood sugar that occurred in the bled rat (curve B) far exceeded that in the control animal, and those that died (curve D) showed a striking fall in blood sugar. If only one suprarenal medulla was removed (curve C), a moderate hyperglycemia occurred immediately after bleeding. The fact that there was some elevation of the blood sugar over the initial value 24 hours after hemorrhage in those animals that survived (curves B and C) was therefore surprising. Liver glycogen levels were therefore measured 24 hours after hemorrhage in the demedullated rats to determine whether the carbohydrate
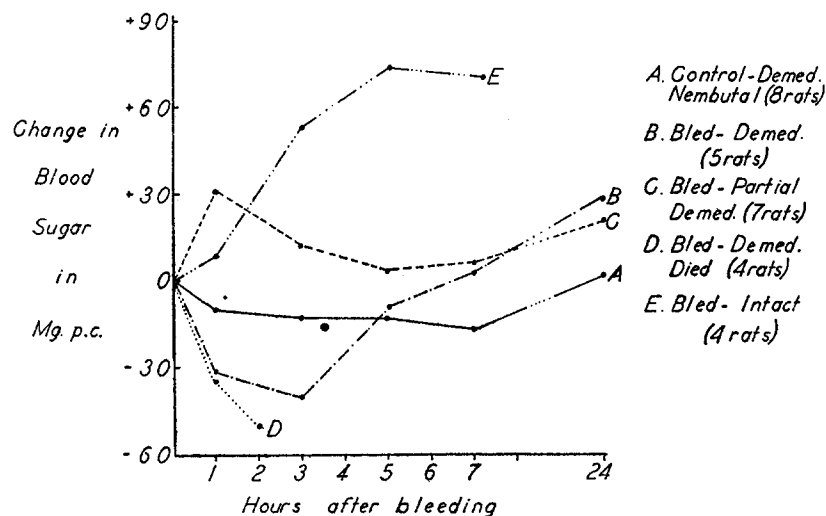

FIG. 8. The blood sugar levels of normal and suprareno-demedullated rats after hemorrhage.

stores were similarly elevated. However, the liver glycogen content of 9 bled animals was not found to be significantly different from 8 control animals fasted a similar length of time, although the former showed a much greater variation in glycogen. An explanation of the discrepancy in blood sugars was found, however, at another source. Analysis of the distribution of glucose between cells and plasma in the rat revealed that the cells contained on the average 60 per cent as much glucose as the plasma. The rise in sugar can therefore be accounted for largely by the increase in the proportion of plasma in the blood 24 hours after bleeding.

DISCUSSION

The data presented here indicate that during hemorrhagic shock in the rat profound metabolic changes take place. When these data are assembled and

considered in the light of previously published data by different investigators, it becomes possible to interpret them in terms of the effects of decreased circution to various organs on the metabolism of those organs.

It has long been known that the non-protein nitrogen content of the blood rises during shock. Taylor and Lewis (12) described such a rise after hemorrhage in dogs in 1915 and noted that the amino acid nitrogen fraction was elevated. Most authors have attributed the N.P.N. elevation entirely to a decrease in renal function. While this is undoubtedly true in part, the determination of a non-protein nitrogen component, whose blood level is not influenced appreciably by renal function, demonstrates that other factors play a considerable rôle in the reported N.P.N. elevations. The blood amino acids under ordinary circumstances exhibit a remarkable constancy and even when amino acids are fed or injected produce only a very transient change in the blood level (13). In rats we have found that the subcutaneous injection of 120 mg. per 100 gm. body weight of amino acids as a casein hydrolysate rarely causes more than 2 to 3 mg. per cent rise in the blood levels lasting about 1 hr. Within the period of time involved in the experiments just reported, nephrectomy does not influence the blood amino acid nitrogen level. On the other hand, after hepatectomy (14) there is a rapid rise in blood amino acids and in the presence of liver damage there is a diminished tolerance to injected amino acids (15). In any given circumstance the rate of rise in blood amino acids will thus be a function of the ability of the liver to deaminate and on the rate of amino acid formation by protein breakdown.

Since all investigations have shown the liver to be of central importance in determining the level of blood amino acid nitrogen, an explanation for the elevations in blood amino acids noted during shock may also be sought in the state of liver function. It is of interest in this connection that several Russian investigators (16, 17) have stressed the rôle of the liver in shock and one (16) has reported elevated blood amino acid levels in man and dogs in this condition. Our data on the relation of the blood pressure to the time at which the blood amino acid changes take place strongly suggest that the latter are secondary to circulatory changes, *i.e.*, decreased blood supply to the liver.

Of all organs, the liver is peculiar in that it receives its major blood supply by vein (18). Moreover, since it has both an arterial and a venous inflow which must eventually meet and the blood be discharged through the hepatic vein, a complicated anatomical arrangement is necessary to equalize the pressures in the two systems. The circulation to the liver and within the liver is under neural control, and can be considerably influenced by such substances as adrenalin, acetylcholine, and pitressin and by changes in arterial and portal pressure. McMichael has studied this problem in some detail (19–21). This worker has estimated that under normal circumstances the portal vein supplies 80 per cent of the oxygen to the liver. Moreover, in an animal with falling

blood pressure, the portal blood becomes progressively unsaturated with oxygen so that at low blood pressure levels the portal blood oxygen saturation may be reduced to half the normal value. This observation combined with that of Blalock and Levy (22) who have reported a 53 per cent decrease in portal blood flow in dogs after a hemorrhage which reduced the blood pressure to between 80 and 100 mm. Hg, serves to emphasize the degree of anoxia to which the liver may be subjected during shock. Dr. H. Harrison in this department has also found a marked decrease in the portal oxygen saturation to occur during hemorrhage in rats.

Unpublished work from this laboratory has shown that the *in vitro* oxygen consumption of liver slices taken from shocked rats may be sharply reduced and that there is a close correlation between the elevation of blood amino acids and the depression in oxygen consumption by the liver slice. This and the observations that the blood fibrinogen and prothrombin (23) are reduced and that plasma protein regeneration is impaired (24) after shock are further evidences of a decrease in liver function during shock. Moreover, the often reported phenomenon that once shock has supervened no further sodium pentobarbital anesthesia is necessary is undoubtedly due in part to the fact that this barbiturate is normally detoxified by the liver. However, it should be mentioned that not all hepatic functions are equally affected by anoxia, for Tanturi and Ivy (25), have shown that bile formation continues even after marked impairment of hepatic circulation. Furthermore, as will be reported later, the observed elevations in blood amino nitrogen cannot be accounted for by hepatic failure alone. Studies on shock in the eviscerated (hepatectomized) rat have shown that there is also an increased rate of protein breakdown in the periphery (26). Similarly, while hepatic insufficiency is undoubtedly a factor in the tendency to hypoglycemia reported here, studies on the eviscerate shocked rat show that an increased rate of glucose utilization by the peripheral tissues is probably of at least equal importance. That the blood amino acid rises are not peculiar either to hemorrhagic shock alone or to the rat is indicated by the preliminary experiments in which the same changes have been observed by us after trauma (leg ligation) in the rat and in one case of clinical shock, and by Mylon, de Sütö-Nagy, and Winternitz in the dog after hemorrhage and leg ligation (23).

The hyperglycemia commonly reported during shock is undoubtedly due to the discharge of epinephrine and is most apt to be manifest when studied in either fed animals or fasted animals previously fed a high protein diet so that they have high liver glycogen levels. When either the suprarenal medullae are removed or the initial liver glycogen level is reduced, the hyperglycemia is completely or largely abolished. Moreover, even in the presence of the suprarenal medullae and a high liver glycogen, terminal hypoglycemia has been noted (27). The progressive fall in blood sugar in the suprareno-demedullated

rat would seem to have its explanation in an increased rate of glucose utilization and a decrease in liver function, as suggested above. The lactate rise, which has been reported many times before, and the pyruvate elevation, previously noted by Govier and Greer (27) in dogs after hemorrhage, would appear to be related to the effects of decreased oxygen supply on the peripheral tissues and the liver. This is at present the subject of more detailed analysis.

From the material presented here it is suggested that the blood chemical changes described in the rats brought into shock by hemorrhage are due on the one hand to a decrease in hepatic function resulting from early anoxia of the liver and on the other to the effects of anoxia on the peripheral tissues causing an enhanced rate of protein breakdown and glucose utilization. In addition, the deficient oxygen supply results in an accumulation of lactate and pyruvate in the blood and tissues.

SUMMARY

During and following the production of shock by hemorrhage in the normal, suprareno-demedullated, and suprarenalectomized rat, the following significant changes in amino acid and carbohydrate metabolism have been observed.

1. In the intact, suprareno-demedullated, and suprarenalectomized rat there is a progressive rise in the whole blood and plasma amino acid nitrogen levels during and after a fatal, shock-inducing hemorrhage. The rate of rise varies inversely with the survival time. In animals surviving the hemorrhage there is little or no elevation in whole blood amino acid levels during the 8 hours following hemorrhage, and a decrease in 24 hours due to hemodilution. The plasma amino acids, however, rise slightly.

2. The blood amino acid nitrogen elevation occurs only after the blood pressure has fallen to between 85 and 90 mm. of Hg.

3. The blood keto acids, as pyruvate, and the blood lactate become elevated during shock in the normal, suprareno-demedullated and suprarenalectomized rat.

4. In the normal fasted rat with low liver glycogen stores the blood sugar may rise moderately or may not rise at all during hemorrhagic shock. In animals with high liver glycogen levels (fed rats or fasted rats previously fed high protein diets) shock generally induces a marked hyperglycemia. In both groups hypoglycemia may occur terminally.

5. In the suprareno-demedullated and suprarenalectomized rats shock is always accompanied by a fall in the blood sugar.

6. There is no significant difference between the liver glycogen levels of suprareno-demedullated rats fasted 48 hours and those similarly fasted but surviving 24 hours after a hemorrhage.

The blood chemical changes have been interpreted as due to a decrease in hepatic function resulting from early anoxia of the liver and to the later effects

of anoxia on the peripheral tissues causing an increased rate of protein break-down and of glucose utilization and an accumulation of lactate and pyruvate in the blood and tissues.

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