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Elevation of Plasma Amino Nitrogen as an Index of the Gravity of Hemorrhagic Shock.*

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In a previous report[1] it was shown that a rise in the amino nitrogen content of blood and plasma was a characteristic feature of the later stages of peripheral circulatory failure. This increase in amino nitrogen was attributed to the combined effects of an increased rate of protein breakdown in the peripheral tissues and a decreased ability of the liver to dispose of amino acids. In that report it was shown that the change in amino nitrogen occurred primarily in the plasma, and the data suggested that the amino nitrogen content of the plasma might be useful as a prognostic index in shock. The present report provides additional data on the distribution of amino nitrogen between the erythrocytes, plasma and whole blood during hemorrhage and on the close correlation between the rise in plasma amino nitrogen and mortality following hemorrhage.

Methods. Male rats of the Sprague-Dawley strain, weighing 180-250 g, were used. The animals were fasted 24 hours before the start of bleeding. Amino nitrogen of whole blood, plasma and sedimented red cells was determined by the method of Frame, Russell and Wilhelmi[2] and hematocrits by the method of Meyerstein.[3]

Results. The effect of hemodilution on whole blood amino nitrogen. Amino nitrogen distributions between whole blood, plasma and red cells were determined in a series of 4 rats during hemorrhage, in order to establish which phase most accurately indicated the state of shock. The rats were bled from the tail 2.5 ml per 100 g of body weight in 1 hour and then returned to their cages, where they were allowed water *ad libitum*. One hour later an additional 1 ml was removed, and every 2 hours thereafter 1 ml of blood was taken until the 4 rats died 13.5, 14, 21, and 21.5 hours respectively from the onset of bleeding. By this method a large degree of hemodilution could develop before fatal shock occurred. Fig. 1 illustrates the changes in one rat. The data from the other 3 animals were entirely comparable. It will be noted that until the twelfth hour the whole blood amino nitrogen paralleled the hematocrit while the red cell and plasma amino nitrogen showed little change until after the tenth hour, when rises occurred. The sharp rise in plasma amino nitrogen which took place when severe shock developed after the twelfth hour was then accurately reflected in the whole blood amino nitrogen, since the hematocrit remained low, and the red cell amino nitrogen showed no significant change. These results show that fluctuations in the hematocrit cause changes in the whole blood amino nitrogen content solely because the red cells contain 2.5 to 3 times as much amino nitrogen per unit of volume as does the plasma. For prognostic purposes, therefore, it seemed best to depend on plasma amino nitrogen concentrations.

Rise in plasma amino nitrogen as an index of the gravity of shock. A series of 74 rats kept in a constant temperature room at

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1 Engel, F. L., Winton, M. G., and Long, C. N. H., *J. Exp. Med.*, 1943, **77**, 397.

2 Frame, E. G., Russell, J. A., and Wilhelmi, A. E., *J. Biol. Chem.*, 1943, **149**, 255.

3 Meyerstein, W., *J. Physiol.*, 1942, **101**, 1.

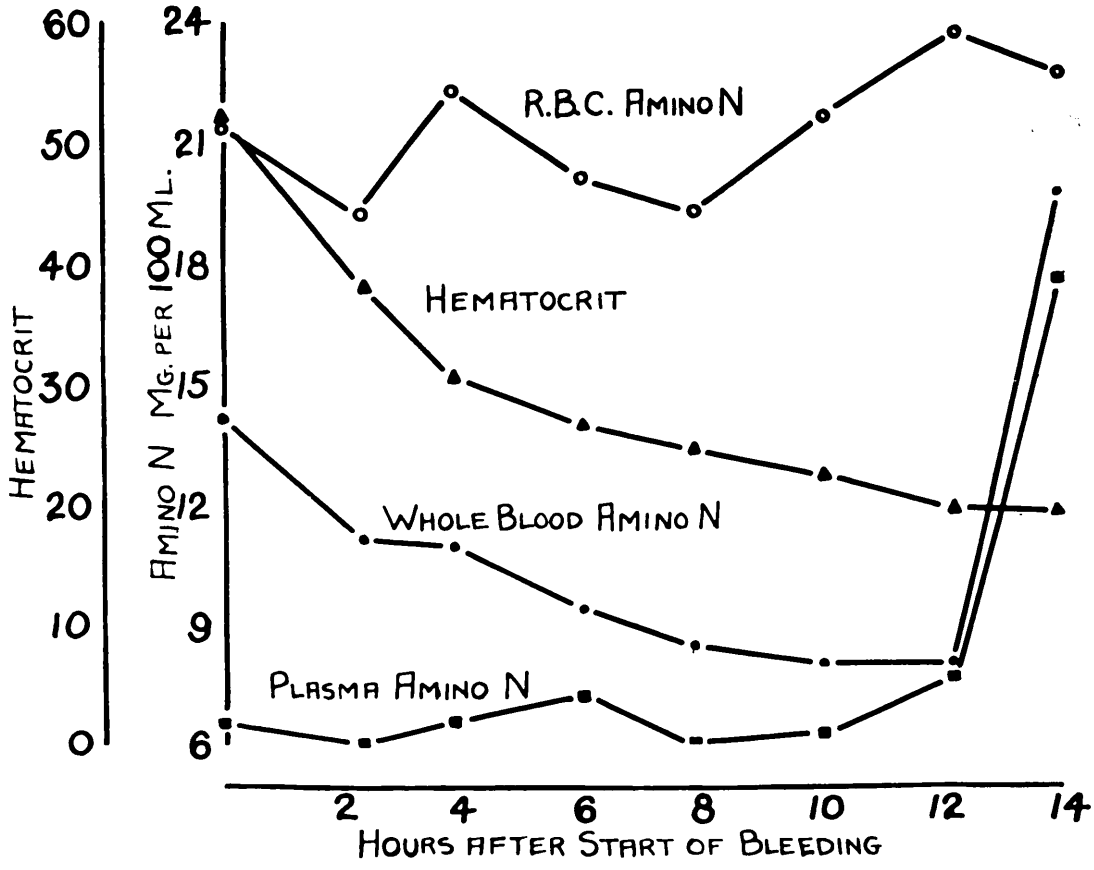

FIG. 1.
The hematocrit and amino nitrogen levels of the whole blood, R.B.C., and plasma of a rat during the course of a prolonged fatal hemorrhage.

83-85° F were suspended in slings according to the technic of Haist and Hamilton[4] and bled from the tails. A volume equal to 2.5 to 2.7 ml per 100 g of body weight was removed over the course of one hour, and plasma amino nitrogen determinations were made at the beginning and the end of the bleeding. No further bleeding was done, and the animals were observed until they died, or for 24 hours if they survived. No water was allowed. A high mortality rate could be expected at the high environmental temperature used. Advantage was taken of the wide variation in survival characteristic of bleeding experiments of this type, in order to correlate the outcome with changes in plasma amino nitrogen. Among those that failed to survive, death occurred from 2 to 140 min after the end of bleeding with an average survival time of 35 min, but there was no correlation between the length of survival and the degree of rise in plasma amino nitrogen during the hour of bleeding. The results in Table I show, however, that there was a good correlation between the proportion of animals surviving and the amount of change in plasma amino nitrogen. When there was a rise of 1.0 mg % or more in amino nitrogen during the hour of bleeding, death invariably occurred if no treatment was instituted. Lesser rises give no sure indication of the outcome but may be useful in indicating a trend toward survival or death if the animals must be sacrificed for experimental purposes.

Discussion. The second group of experiments cited above shows that a rather small rise in plasma amino nitrogen after hemorrhage is a reliable sign that the animal is not

[4] Haist, R. E., and Hamilton, J. I., *J. Physiol.*, 1944, **102**, 471.

TABLE I.
Relation Between the Change in Plasma Amino Nitrogen During Bleeding and the Survival of Rats.

Change in plasma amino nitrogen,* mg %	No. rats	Died	Survived	% died	Survival time in min.†
—1.0 to —0.1	9	3	6	33	55 to 140 (Avg 84)
0 to +0.5	20	11	9	55	5 to 72 (Avg 36)
+0.5 to +1.0	19	14	5	74	6 to 65 (Avg 33)
+1.0 to +5.6	26	26	0	100	2 to 120 (Avg 35)
	74	54	20	73	

All animals bled 2.5-2.7 ml per 100 g of body weight in 1 hour.
* Change in plasma amino nitrogen from beginning to end of hemorrhage.
† Survival time of animals that died only are included. Time in minutes after end of bleeding.

going to survive unless treatment is instituted. The course of the changes in blood amino nitrogen in the single rat cited in Fig. 1 indicates that small but significant increases in plasma amino nitrogen might be missed if the determinations are made only on whole blood. Due to the changes in hematocrit and red cell amino nitrogen in the first 2 hours after start of bleeding, the whole blood amino nitrogen would change by —1.8 to +1.1 mg % for a rise in plasma amino nitrogen of from 0.8 to 5.6 mg %. (It may be noted that the plasma amino nitrogen of the rat represented in Fig. 1 fell slightly by the second hour after start of bleeding, and that this animal survived not only the initial bleeding but several additional hemorrhages. This is consistent with the relation between change in plasma amino nitrogen and likelihood of survival illustrated by the data of Table I.) A rise in plasma amino nitrogen does not mean that the animal cannot be resuscitated if adequate therapy is instituted. Many rats have recovered after receiving transfusions at a time when their amino nitrogen levels were several times normal and the animals appeared moribund.

The change in plasma amino nitrogen levels during hemorrhage and other shock-inducing procedures is recommended as a reliable method of estimating the severity of shock before therapy is instituted or if it is desired to sacrifice the animal for other studies at any given time after the shock-inducing measure has been instituted. Russell *et al.*[5] made use of a similar procedure when studying the oxygen consumption of liver slices at various times after hemorrhage in rats.

Summary. Serial determinations of the hematocrit and the whole blood, plasma and red cell amino nitrogen concentrations were carried out during prolonged hemorrhage and shock in rats. It was shown that, due to the high concentration of amino nitrogen in the red cell, as compared to the plasma, the whole blood amino nitrogen fluctuates with the hematocrit, while the plasma amino nitrogen more closely reflects the degree of shock. In a large series of rats a rise in plasma amino nitrogen of 1 mg % or more after a 2.5 to 2.7 % hemorrhage was invariably associated with a fatal outcome.

[5] Russell, J. A., Long, C. N. H., and Wilhelmi, A. E., *J. Exp. Med.*, 1943, **79**, 23.