AMINO-ACIDS, SERUM AND PLASMA IN THE REPLACEMENT THERAPY OF FATAL SHOCK DUE TO REPEATED HEMORRHAGE*

AN EXPERIMENTAL STUDY

ROBERT ELMAN, M.D., AND CARL E. LISCHER, M.D.

ST. LOUIS, MO.

FROM THE DEPARTMENT OF SURGERY, WASHINGTON UNIVERSITY MEDICAL SCHOOL, AND BARNES HOSPITAL, SAINT LOUIS, MO.

IN PREVIOUS STUDIES from this clinic it was shown[8, 9, 11] that the amino-acids of hydrolyzed protein are effective as a parenteral method of correcting hypoproteinemia of nutritional origin. In view of the obvious practical advantages of such solutions over plasma, it was natural to try their possible therapeutic value in the acute hypoproteinemia which follows severe hemorrhage. In this communication are described experimental studies on fatal shock produced by repeated hemorrhage, in which replacement of the lost blood by an amino-acid mixture seemed to have a beneficial effect. A preliminary note has already been published.[12]

Extensive experiments, of course, have dealt with the production of surgical shock by hemorrhage,[13, 25] many by members of this Association.[1, 17, 20] However, in the present experiments the approach has been, to a large extent, biochemical rather than physical or physiologic. This deserves emphasis, inasmuch, as amino-acid mixtures can in no real sense be classed as a blood substitute because they lack the colloidal properties of blood plasma. The value of such injections, if they have value, must depend upon the ability of the body to use amino-acids to synthesize plasma proteins rapidly, or for nutritive or other metabolic purposes. Nevertheless, comparative experiments were carried out with serum and plasma.

Considerable evidence that protein metabolism is a rapid procedure emerges from the important work[22] with isotopic nitrogen. Other workers[2] have recently shown that similarly labeled amino-acids appear in plasma proteins within an hour after injection. These fundamental studies indicate that it is theoretically possible for injected amino-acids to be made into plasma proteins very rapidly, and thus act as an indirect substitute or supplement to plasma. Undoubtedly, the liver is the key organ in this process and we shall have some evidence to show that this is the case.

EXPERIMENTAL PROCEDURES

Because surgical shock involves so many variables, we adopted a method which seemed to give a relatively uniform response. Instead of basing our experiments on the level of hypotension or the amount of hemorrhage, we subjected dogs to uniform repeated bleedings (10 cc. per kilogram of body weight each hour) until death ensued, and then used the survival time as the basis for measuring the effect of various replacement procedures. Systolic

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blood pressure as well as hematocrit and fractional plasma proteins were also determined. General anesthesia was not used. The effect of changes in posture was avoided by carrying out the entire experiment while the animal was in the supine position.

Unselected mongrel dogs, varying in weight from 7 to 19 kilograms, were given water *ad libitum,* but no food during the 18 hours preceding the beginning of each experiment. A cannula was inserted into the femoral artery and bleeding carried out as rapidly as possible; the calculated amount usually being obtained within a minute or two. The systolic blood pressure was recorded before and after each hemorrhage with a Tycos manometer[7] connected to the same cannula used for bleeding. For hematocrit and fractional plasma protein determinations, samples of heparinized venous blood were taken immediately preceding each hemorrhage. The former was measured after centrifugalization at 3000 R.P.M. for 30 minutes. Supernatant plasma was drawn off; the nonprotein nitrogen estimated by nesslerization, fractionation carried out,[4] and nitrogen determined by a Kjeldahl procedure.[23]

The therapeutic value of the various solutions tested was compared by using them to replace the blood removed after each hemorrhage. The fluids for replacement were sterile, but no attempt at complete asepsis was made during the experiment because of its short duration. The injections were rapid and followed immediately each bleeding, the volume being exactly equal to the volume of blood removed. In most experiments the fluids were injected into the femoral artery through the same cannula used for bleeding, but in a few groups the fluid was introduced into the adjacent femoral vein.

Seven groups of experiments were carried out as follows:

TABLE I

	No. of Dogs	Replacement Fluid
Group I	10	None.
Group II	20	10% glucose in normal saline solution.
Group III	6	Pure amino-acids 5%, glucose in normal saline 5%.
Group IV	20	Protein hydrolysate 5%, glucose in normal saline 5%.
Group V	10	Dog serum diluted with an equal quantity of 10% glucose in normal saline.
Group VI	10	Citrated dog plasma diluted with an equal quantity of 10% glucose in normal saline.
Group VII	10	Heparinized dog plasma diluted with an equal quantity of 10% glucose in normal saline.

The protein hydrolysate* was an enzymatic digest of casein (Amigen) or beef serum. No difference was observed in the results with these two hydrolysates. The pure amino-acids† consisted entirely of essential amino-acids.[21] Both solutions were neutralized with sodium hydroxide to a p_H of 6.5 to 7.5. The nitrogen content of the two was similar, *i.e.,* 0.66 and 0.63 gm. per cent, respectively. Serum was obtained by centrifuging clotted dog blood; in some cases it was fresh and in others it had been stored for two weeks at freezing temperatures before separation; no obvious difference was

* Supplied through the courtesy of Dr. Warren M. Cox, of Mead Johnson and Co.

† Supplied through the courtesy of Dr. D. F. Robertson, of Merck and Co.

observed in the effects of these two methods of preparation. The citrated plasma contained about 0.6 gm. per cent of sodium citrate. To obtain the heparinized plasma one-half cubic centimeter of heparin solution (Lederle) was added to each 1000 cc. of whole dog blood.

It should be pointed out that dilution of whole plasma and serum was necessary, so that the actual amount of plasma protein replaced was roughly the same as that removed. An equal amount of diluting fluid was used because the average red cell volume was 50 per cent. Constancy of the plasma proteins was insured by using pooled plasma from many dogs.

At the termination of each experiment a microscopic section was made of the liver. In a separate series of six dogs, a biopsy of the liver was removed under nembutal anesthesia two hours before the initial bleeding and at death. In three of them glucose was used as replacement, and in the other three hydrolyzed protein.

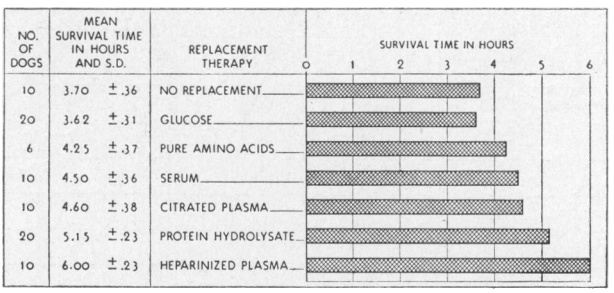

FIG. 1.—Survival-Times in the Seven Groups of Experiments Described in the Text: S. D. = Standard deviation, calculated according to usual statistical formulae. Note that replacement with heparinized plasma and a protein hydrolysate gave the longest survival time, and the relatively poor showing with serum and citrated plasma.

EXPERIMENTAL RESULTS

The first observation of interest was that removal by repeated bleedings of an average of but 43 cc. per kilo of blood led to an invariably fatal outcome; by contrast, survival is frequent when the same amount of blood is removed in a single hemorrhage. This has been the experience of other observers and we have performed single hemorrhages of this magnitude with a mortality of but 20 per cent.[9] Clinical observations are similar, in that patients often survive a single bleeding, but are apt to succumb after subsequent though smaller ones. This is especially true of war wounds, for even if there has only been a single initial hemorrhage, further bleeding is sure to occur during débridement.

Survival Time.—Of greater importance was the observation (Fig. 1) that replacement with 10 per cent glucose in normal physiologic saline had no influence on the survival time, in contrast to the results with the solution containing hydrolyzed protein, in which the survival time was

5.1 hours, as compared with 3.6 hours for the controls.* The experiments with pure amino-acids were only six in number, but the results (survival time = 4.2 hours) were only somewhat better than with glucose (survival time = 3.6 hours), indicating that the greater effect of the hydrolyzed protein on survival time could not have been due to the mere presence of amino-acids *per se*.

Of special significance were the comparable experiments in which serum, citrated plasma and heparinized plasma were used for replacement. The first two fluids produced no greater survival time than was obtained with the

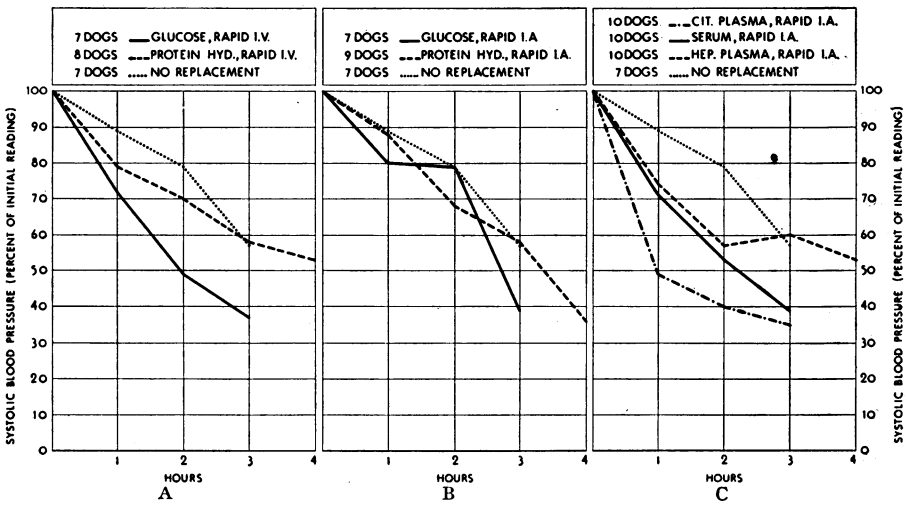

FIG. 2.—Blood Pressure Response: The curves were drawn from mean values in groups of dogs surviving at least three or four hours, but does not include the terminal fall. The curve in which there was no replacement is shown in all of the above three graphs.

A. Note pronounced fall during intravenous replacement with glucose as compared with hydrolyzed protein.

B. With intra-arterial replacement the difference noted in A is the same, but not as pronounced.

C. Note the precipitate early fall with citrated plasma. Serum showed the same though somewhat delayed drop. Heparinized plasma, after two hours, maintained a good level of pressure.

hydrolyzed protein. The heparinized plasma, on the other hand, resulted in the longest survival time (six hours).

Of some interest is the fact that no differences were observed in the survival time between experiments in which the fluid was replaced intravenously as compared with those in which they were injected intra-arterially. This topic has been the subject of previous investigations.[6, 15, 16]

Blood Pressure Response.—A progressive fall in blood pressure was observed in each group of experiments as shown in Figure 2. In general, the behavior of the blood pressure was somewhat variable, an observation made by others. In many instances the drop after each hemorrhage was pronounced but recovery good, whereas, in others the blood pressure was

* That this difference is statistically significant is shown by dividing the difference of the means by the standard deviation of the difference. When the figure thus obtained is used in consulting statistical tables, it becomes a certainty that this difference is not a chance phenomenon.

maintained rather well but then dropped abruptly before death. Nevertheless, the average values obtained in similar groups of experiments show significant variations which in most instances can be correlated with the survival time. For example, the greatest blood pressure drop was exhibited by the dogs in which citrated plasma was used, whereas, with the heparinized plasma the blood pressure was sustained after a drop in the first two hours. Moreover, the hydrolyzed protein was followed by a definitely higher blood pressure than similar experiments with ten per cent glucose, particularly when replacement was intravenous rather than intra-arterial. Quite surprising was the fairly good blood pressure, until the terminal fall, in dogs bled with no replacement.

Red Cell Volume and Plasma Proteins.—A progressive fall in red cell volume, plasma albumin, and globulin was observed in all experiments, although there were definite and significant differences between the different groups. For purposes of simplification, presentation of this data is limited to that shown in Table II. Therein, are figures representing the observations made three hours after the beginning of the experiment, *i.e.*, after each animal had lost 30 cc. per kilo, and just before the fourth hemorrhage was carried out. The numbers refer to the fall in value expressed as a percentage of the initial determination. For example, the experiments in which pure amino-acids were used for replacement at three hours showed a drop in hematocrit to 98 per cent, in albumin to 83 per cent, and globulin to 88 per cent of the initial value.

TABLE II

	Hematocrit	Albumin	Globulin
Pure amino-acids	98	83	88
No replacement	94	90	94
Hydrolyzed protein	88	81	75
Glucose	83	74	71
Heparinized plasma	79	83	81
Citrated plasma	75	77	79
Serum	85	85	94

The importance of this data on plasma proteins and red cell volume lies in the fact that they measure the factors influencing fluid shift and colloidal osmotic pressure, which, as discussed in a previous paper,[9] control these compensatory mechanisms following hemorrhage. In other words, restoration of blood volume from extracellular fluid (hemodilution) is indicated by the fall in the hematocrit, while the addition of the two plasma proteins to this diluting fluid, particularly albumin, which contributes 85 per cent of the colloidal osmotic pressure of the blood, is indicated by the comparative changes in their relative concentrations.

Pathology.—At autopsy, the bloodless condition of the tissues was a uniform finding. The viscera were pale and in no instance was any engorgement or congestion of the intestines observed. Study of microscopic sections of the liver were of greatest interest, and showed in general a shrinking or diminution of stainable cytoplasm. This was less pronounced in the dogs

receiving hydrolyzed protein as compared with those getting glucose as replacement (Figs. 4, 5 and 6). The significance of these findings is discussed later on.

FIG. 3

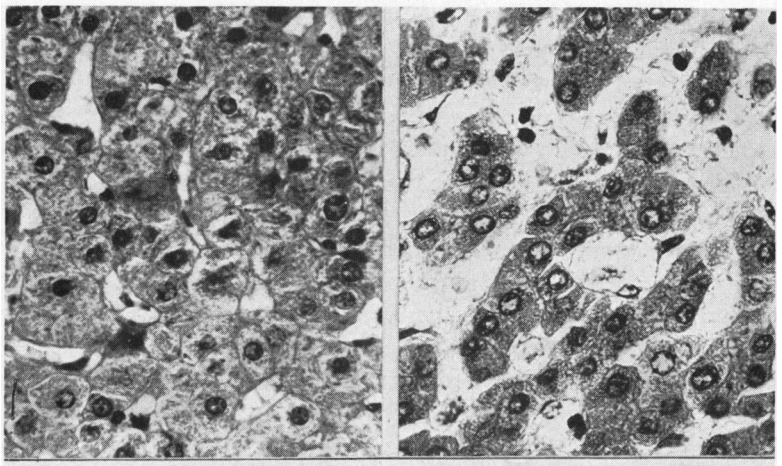

FIG. 4

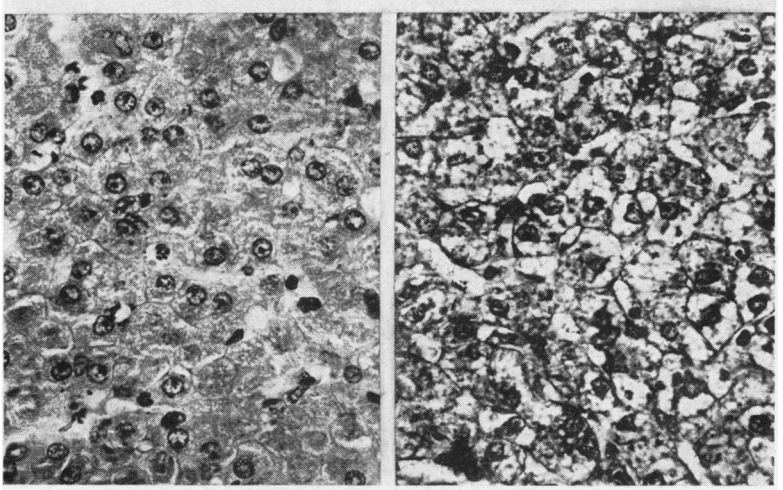

FIG. 3.—Photomicrographs (× 570) before and after a severe hemorrhage. The patient was a 14-year-old female; a splenectomy carried out. The section at the left shows the condition of the liver as revealed in a biopsy taken at this time. The section at the right is the autopsy specimen removed 6 hours later, death being due to a large intra abdominal hemorrhage. Note the atrophy, the liver cords on the right indicating a depletion of hepatic cytoplasm. The sinusoids are correspondingly dilated.

FIG. 4.—Photomicrographs (× 570) of liver sections obtained at autopsy in fatal experimental hemorrhages in dogs. In the left the bled blood had been replaced by a solution containing hydrolyzed protein, on the right by glucose. Note the loss of stainable hepatic cytoplasm (vacuolization) on the right as compared with the relatively normal liver cords on the left.

COMMENT.—Two features of the present experiments should be emphasized. First, general anesthesia was not used, thus, as pointed out previously,[9] eliminating certain complicating factors in the normal response to hemorrhage. Second, a fatal outcome was invariable in each experiment without altering the details of the procedure, thus avoiding any subjective influence on observations.

The results with serum and plasma replacement have an immediate practical bearing on the use of these fluids in the treatment of human surgical shock. The poor showing of citrated as compared with heparinized plasma is obvious on comparing the blood pressure response as well as the survival time. This confirms experiments performed by others.[3, 14] The fact that citrated plasma produced changes in red cell volume and in plasma proteins which were similar to those with heparinized plasma (Table II) suggests that citrated plasma was just as effective in controlling fluid shifts and colloidal osmotic pressure. It would seem, therefore, that a toxic effect of sodium citrate may have been responsible. That serum was no better than citrated plasma is more difficult to explain, although there is some evidence that serum, under certain conditions at least, is toxic when used instead of plasma.[19] Serum, moreover, behaved unlike plasma in that the changes in hematocrit and plasma protein were somewhat different as indicated by the findings in Table II. For example, the albumin fell more than the globulin, suggesting some loss of this fraction from the circulation. In experiments by others,[14] serum was also found to be inferior to heparinized plasma, in replacement of large single hemorrhages in the dog. Further study of the effect of serum injections seems necessary.

As to the beneficial effects of hydrolyzed protein on survival time and on blood pressure, the most obvious explanation is that they were utilized in the synthesis of serum albumin, thus enabling the body to withstand the effects of further hemorrhages. Study of the hematocrit and plasma protein determinations does seem to justify such an inference. Thus, perusal of Table II shows a slight but definite difference in the behavior of albumin and globulin. It will be noted that the fall in albumin was relatively less (as compared with the globulin) in the experiments in which hydrolyzed protein as compared with those in which glucose was injected. This suggests that more albumin was added to the diluting fluid in the experiments in which hydrolyzed protein was used.

A contrasting result on red cell volume and plasma proteins is apparent in the experiments in which pure amino-acids were used and the ones in which no replacement was carried out. In both of these groups there was very little hemodilution, which means but slight restoration of blood volume. For this reason, the relatively slight fall in protein was of little significance as far as restoration of lost plasma proteins is concerned. Yet the albumin dropped much more than the globulin, indicating loss rather than addition of albumin to the circulating blood during the rather insignificant inflow of fluid. In other words, the compensatory mechanisms controlling fluid shifts seemed less active than in the other experiments. Why the pure amino-acids proved less effective than the hydrolyzed protein is probably due to the fact that they differed significantly in composition from the latter in that they contained no polypeptides and only the essential amino-acids, most of which were synthetic and, therefore, available only as racemic mixtures. Because of the last factor, a majority of the amino-acids were present in the unnatural form, which are probably biologically ineffective.

In evaluating the results with hydrolyzed protein, the biopsy studies of the liver are of considerable interest. In general, they show changes indicating that the liver contained a higher protein content in the experiments with

FIG. 5

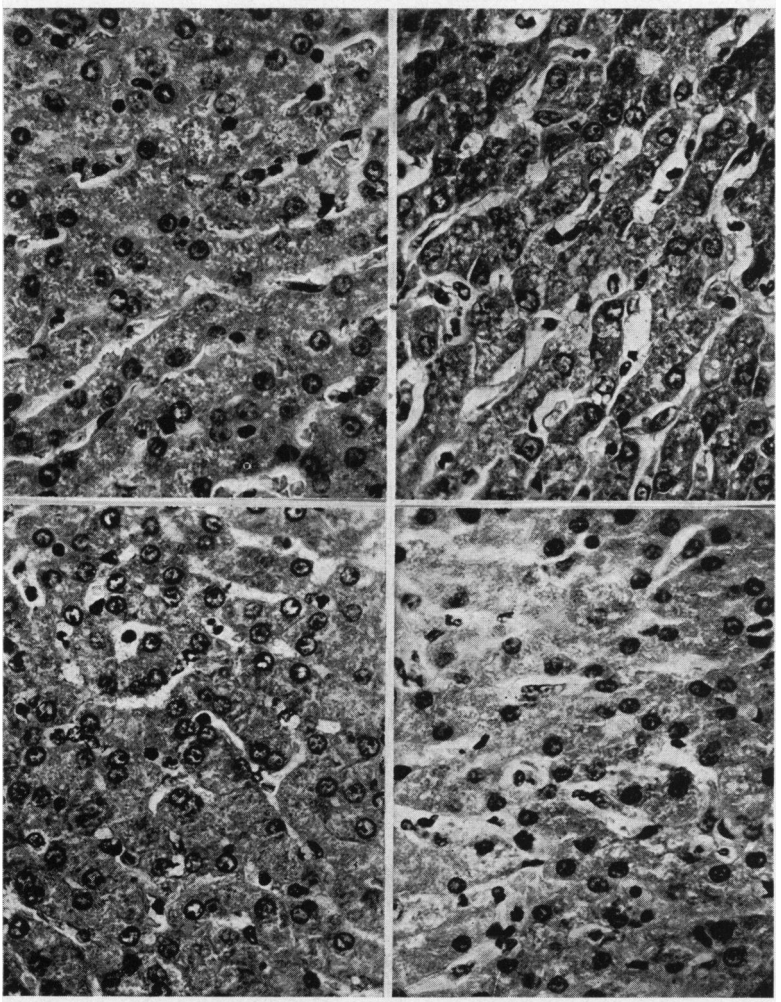

FIG. 6

FIG. 5.—Photomicrographs (× 570) of liver sections before and after repeated hemorrhages; replacement with glucose. This experiment differs from the others in that nembutal anesthesia had to be given to get the control biopsy. Nevertheless note the atrophy of the liver cords as well as vacuolization of the cytoplasm on the right as compared with the control on the left. This change is to be contrasted with Text Fig. 6 in which hydrolyzed protein was used for replacement.

FIG. 6.—Photomicrographs (× 570) of liver sections before and after repeated hemorrhage; replacement with solution containing hydrolyzed protein. Note the relative absence of atrophy and vacuolization on the right as compared with the changes observed in Text Fig. 5.

hydrolyzed protein than in those with glucose (Figs. 5 and 6). This interpretation is based upon many previous studies in which the amount of protein in the liver seemed to be correlated with the stainable protoplasm.[10, 11] Corroborative, are other observations[18] in which stained droplets in the liver,.

apparently protein in nature, disappeared following experimental hemorrhage. Moreover, we have observed, in a human, what seems to be a loss of hepatic cytoplasm as a result of a fatal hemorrhage (Fig. 4). Further studies, however, will be necessary before this correlation rests on more secure foundations.

That the speed of the intravenous injection has some influence on the results also seems evident. Thus, many of the experiments described herein were repeated, giving the replacement fluid slowly by intravenous drip over the course of the hour between hemorrhages instead of rapidly following each bleeding. The results were remarkably similar to those reported above, with the exception of glucose and heparinized plasma, which resulted in a slightly longer mean survival time when given slowly intravenously. That the manner of replacement has an important influence was also noted in a report[24] of shock induced in dogs by the tourniquet method; while fatalities were successfully combated by repeated small plasma transfusions, the slow injection of plasma for 30 to 50 minutes following release of the tourniquet resulted in shock and death, although the total amount of plasma given to the two groups was the same. Reasons for this difference were not found on analysis of the data on hematocrit, plasma protein, hemoglobin and blood volume.

A comment should be made in regard to amino-acid mixtures made by hydrolyzing protein, *i.e.*, any protein may be hydrolyzed to any degree. The product used herein was hydrolyzed to about 70 per cent of completion; in other words, 30 per cent of the protein molecule was still in the form of amino-acid aggregates or small polypeptides. The larger these aggregates the more they approach the properties of protein and the more likely they are to possess colloidal properties. These considerations may eventually prove of practical importance, inasmuch, as slightly hydrolyzed proteins may be of sufficient molecular size to exhibit the properties of plasma proteins and yet may have lost any anaphylactic or antigenic properties possessed by the intact molecule. Moreover, no one knows how small the hydrolyzed protein molecule must be before it can be utilized as such by the body in the synthesis of other proteins or for other metabolic needs. Further investigation would seem to be worth while.

Certain additional clinical implications may be drawn from the present data. Hemorrhage, in many instances, will be accompanied by considerable trauma. Whether this be the result of an accident or a planned operative procedure, there is an increased nitrogen loss indicating excessive tissue protein breakdown.[5] When there is acute loss of protein, as in hemorrhage, the body stores will attempt to replenish this loss at their own expense, thus adding to the depletion of tissue protein. To replace protein lost from the circulating blood is a simple problem and can be accomplished directly with whole blood or plasma. On the other hand, correction of tissue protein depletion is easier and more direct with amino-acids than with plasma, even when large amounts of the latter are given. Thus, in the severely wounded or severely depleted individual nitrogen metabolism can be restored to normal more readily by using protein hydrolysates, thus, accelerating recovery. Moreover, if whole

blood or plasma is not available it would seem that protein hydrolysate is preferable to the use of ordinary crystalloid in the treatment of shock due to hemorrhage.

SUMMARY

Fatal surgical shock in unanesthetized dogs followed bleeding ten cubic centimeters per kilo of body weight, every hour, the mean survival time being 3.6 hours. There was a progressive fall in blood pressure, in red cell volume, and in plasma albumin and globulin in all experiments. If the bood removed each time was immediately replaced by the same volume of various solutions, significant differences were observed as follows:

The survival time was unchanged with glucose in saline, increased to 4.2 hours with pure amino-acids, to 5.15 with hydrolyzed protein. With citrated plasma or serum survival time was but 4.5 and 4.6 hours, whereas with heparinized plasma it was 6.0 hours.

The fall in blood pressure was greater with citrated plasma and serum than with heparinized plasma, whereas, hydrolyzed protein produced less hypotension than glucose.

Study of the changes in red cell volume and in plasma proteins gives some indication that the amino-acids of hydrolyzed protein were converted into plasma albumin. Histologic study of the liver suggests that protein is lost from the hepatic cytoplasm in hemorrhage, and that injecting hydrolyzed protein replenishes this loss, as compared with experiments in which glucose was used.

It may be inferred that in shock due to repeated hemorrhage a solution containing the amino-acids and peptides of hydrolyzed protein has a beneficial influence as compared with glucose and that heparinized is far superior to citrated plasma. Various implications of the problem of shock from repeated hemorrhage are discussed.

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DISCUSSION.—Dr. Robert Elman (St. Louis, Mo.): The general phenomenon of plasma protein regeneration, particularly by dietary means, has, of course, been extensively studied, especially by the Rochester School, under Doctors George Whipple and S. C. Madden. What we have shown, I believe, is that this regeneration occurs rapidly enough after repeated acute blood loss to have a beneficial effect, provided a proper mixture of amino-acids is injected. Such solutions may, therefore, have a place in the treatment of shock, insofar, as they can be transformed into plasma proteins, thus enabling the body to withstand further loss of blood, which might otherwise prove fatal. This may at least spare the need for much of the plasma which would be required after the initial hemorrhage.

I would like to pursue the biochemical point of view a little further. It seems clear that much of the problem in the treatment of shock following hemorrhage concerns the need for rapid restoration of plasma proteins lost in the bleeding. Now plenty of fluid is available in the body to restore blood volume spontaneously; actually about 20 liters are normally present in the extracellular spaces of an average-sized adult, and it is quickly mobilized after hemorrhage. Unfortunately, however, this fluid contains practically no protein and this is the reason why its compensatory effect is inadequate. True enough, plasma proteins are regenerated eventually, but without outside aid, this requires days instead of hours. Now, if a soldier dies after a total loss of two liters of blood, one could explain the fatality by saying that the patient urgently needed 60 gm. of plasma protein; and that the inability of the body to supply it may be described by a well known phrase—"too little and too late." Yet, consider how strange this is: Sixty gm. of protein needed in the blood stream, while kilograms of tissue protein are present everywhere, without being used. Well might one quote the words of the Ancient Mariner: "Water, water everywhere, nor any drop to drink." According to this line of reasoning experimental studies aiming to find means to supplement or supplant the use of plasma might well be directed toward the possibility of accelerating endogenous plasma protein replacement. Indeed, it may not be too farfetched to look forward to such acceleration from the tremendous tissue protein stores by merely injecting appropriate enzymes or catalysts.

DR. LESTER R. DRAGSTEDT (Chicago): This paper by Doctors Elman and Lischer presents a number of interesting and puzzling facts. I should have anticipated that a single massive hemorrhage would be more apt to be fatal than the gradual loss of the same amount of blood because of the time for the compensatory mechanism to become active in the former case. I should like to know if the authors have any explanation for this contrary finding.

The better results from the use of protein hydrolysates as compared with glucose, it seems to me is very likely to be explained as they have suggested, by the formation of plasma proteins from the amino-acids supplied. It means a much more rapid manufacture of proteins from amino-acids than I would have believed possible.

I wonder if a possible explanation could be that in the presence of this massive hemorrhage protein reserves have been called out from the tissues and that the amino-acids supplied have furnished material for the more gradual replenishment of the tissue proteins.