

CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

VII. CONCENTRATED HUMAN SERUM ALBUMIN^{1,2,3}

PART I. ALBUMIN IN THE TREATMENT OF SHOCK.

PART II. SAFETY OF ALBUMIN.

PART III. ALBUMIN IN THE TREATMENT OF HYPOPROTEINEMIA.

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INTRODUCTION

Specialized combat units, such as landing parties, paratroops, seabees, and commandos, require fighting equipment and supplies especially adapted to their needs. All supplies should be compact, as transportation space is at a premium in these highly mobile units. The Standard Army and Navy Package of Dried Plasma is bulky, and therefore concentrated human serum albumin was developed (1) to meet the needs of such groups for a concentrated blood derivative.

Although albumin may not be available in civilian practice for some time, this paper summarizes our experience in the hope that it may

serve as the starting point for future studies. This experience has been gained over a two-and-a-half-year period, the first part of which was devoted to an evaluation of albumin as a therapeutic agent in shock,⁷ and the latter part to the testing of each albumin lot for acceptance by the armed forces.

It must be emphasized that the work to be presented represents the cooperative efforts of so many individuals that it would be impossible to name all those whose labors have gone into these studies. Credit must go to the American Red Cross Blood Donor Service, which organized the collection of the blood, from which both human plasma and human serum albumin have been prepared, and to the laboratory workers who carried on the production of albumin during the experimental stages of the program. The large-scale production which has been accomplished was made possible through the development of sound theoretical procedures by those working in the laboratory where the methods originated.

PART I. ALBUMIN IN THE TREATMENT OF SHOCK

A. The theoretical and experimental basis for its use

Serum albumin has at least two known functions: it maintains the colloid osmotic pressure of the blood and plays a rôle in the nutrition of the tissues. We are primarily concerned with

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⁷ The appraisal of albumin was aided greatly by Soma Weiss, R. F. Loeb, A. Blalock, I. S. Ravdin, and O. H. Wangensteen.

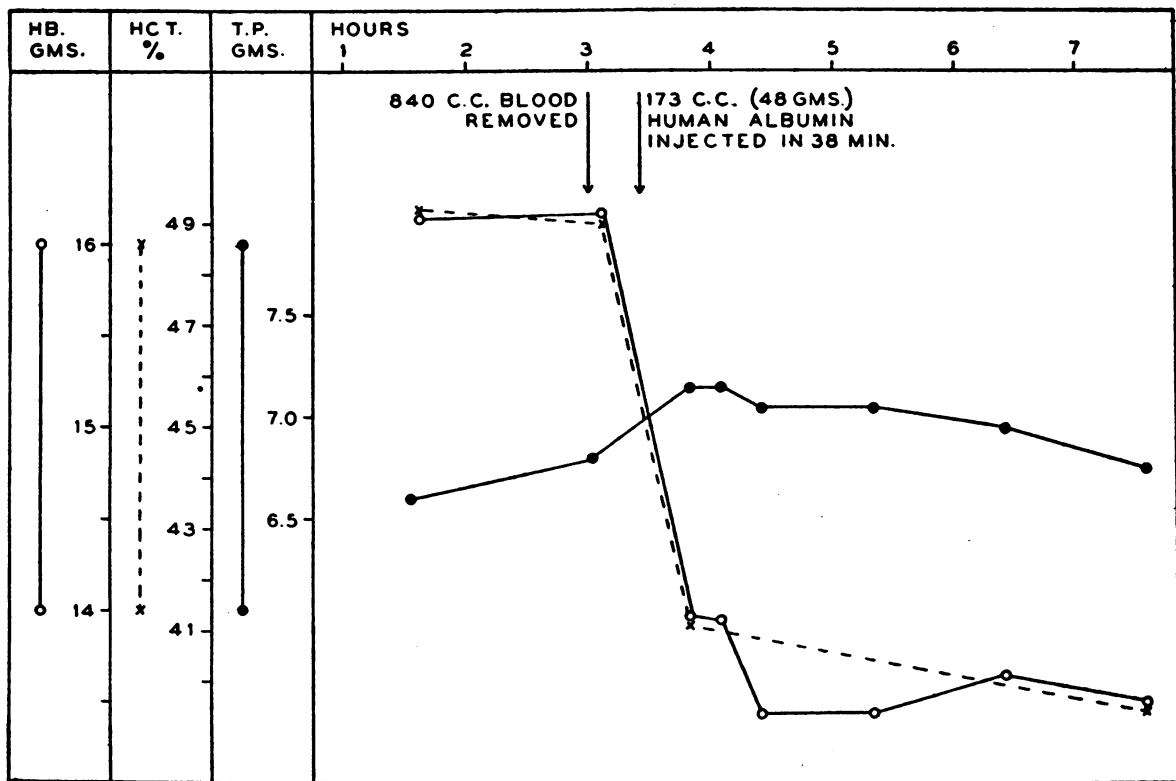


FIG. 1. RESPONSE OF A NORMAL HUMAN SUBJECT TO VENESECTION FOLLOWED BY RAPID INTRAVENOUS INFUSION OF CONCENTRATED HUMAN ALBUMIN (data of Stead and Ebert)

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the former in this section. Although it comprises but about 60 per cent of the plasma proteins, albumin accounts for approximately 80 per cent of their colloid osmotic pressure (2). This high osmotic activity makes serum albumin an important factor in the regulation of the volume of circulating blood.

Certain properties of serum albumin have much to do with its practical therapeutic usefulness. Not only is it osmotically active, but also extremely stable, and very soluble in water or in solutions of various crystalloids. Dispensed as a concentrated solution, albumin takes up much less space than a comparable amount of dried plasma and is ready for instant injection without reconstitution. As with plasma, no cross-matching is necessary. Its stability is an important asset for military use, permitting transportation over long distances without refrigeration. The relative symmetry of the albumin molecule im-

parts a low viscosity to albumin solutions; in fact, a 25 per cent solution is approximately isoviscous with whole blood (2).

The proposal to use concentrated albumin in the treatment of shock depended on the premise that, due to its high colloid osmotic pressure, it would increase the circulating blood volume by drawing on the tissue fluids of the patient. Preliminary experiments in human subjects by Stead and Ebert (3) showed that this actually was the case. Measurements made by Scatchard, Batchelder, and Brown (4) indicated that each gram of albumin should hold 18 cc. of fluid in the circulation by virtue of its colloid osmotic pressure. In conjunction with their studies, some of us (5) made measurements of the plasma volume increase produced by albumin injection following rapid blood depletion by venesection. The average increase (one hour after injection) was 17.4 cc. per

gram, although there was considerable variation between individual experiments. This corresponded closely with the 18 cc. expected and justified the belief that concentrated albumin would exhibit the same type of osmotic behavior *in vivo* as *in vitro*. Consequently, 25 grams of albumin, representing the osmotic equivalent of 500 cc. of citrated plasma, was taken as the standard dose.

The immediate response of patients to an injection of concentrated albumin is quite uniform. There is a rapid fall in hemoglobin concentration and hematocrit reading, indicating the transfer of extravascular fluid into the circulation. Unlike the hemodilution due to an injection of saline solution, that following albumin is not accompanied by a fall in the serum protein concentration. In Figure 1, the response of a patient to concentrated albumin after blood loss is shown. This illustrates the

rapid hemodilution which occurs, associated with a slight increase in serum protein concentration. Most of the effect of the albumin is exerted during the period of injection, but for the next few hours, there is a gradual decline in the values for hemoglobin and hematocrit, with corresponding return of the serum protein value to normal. Figure 2 illustrates the difference in hematocrit response after acute blood loss following the injection of concentrated albumin or of saline solution, as compared to the changes occurring in an untreated control subject.

When concentrated albumin is injected into a patient with a normal blood volume, a similar immediate hemodilution occurs. However, in such a patient the hemodilution is transient, and the values for hemoglobin and hematocrit return toward normal in a few hours. This contrast in response between patients with normal and depleted blood volumes is shown in

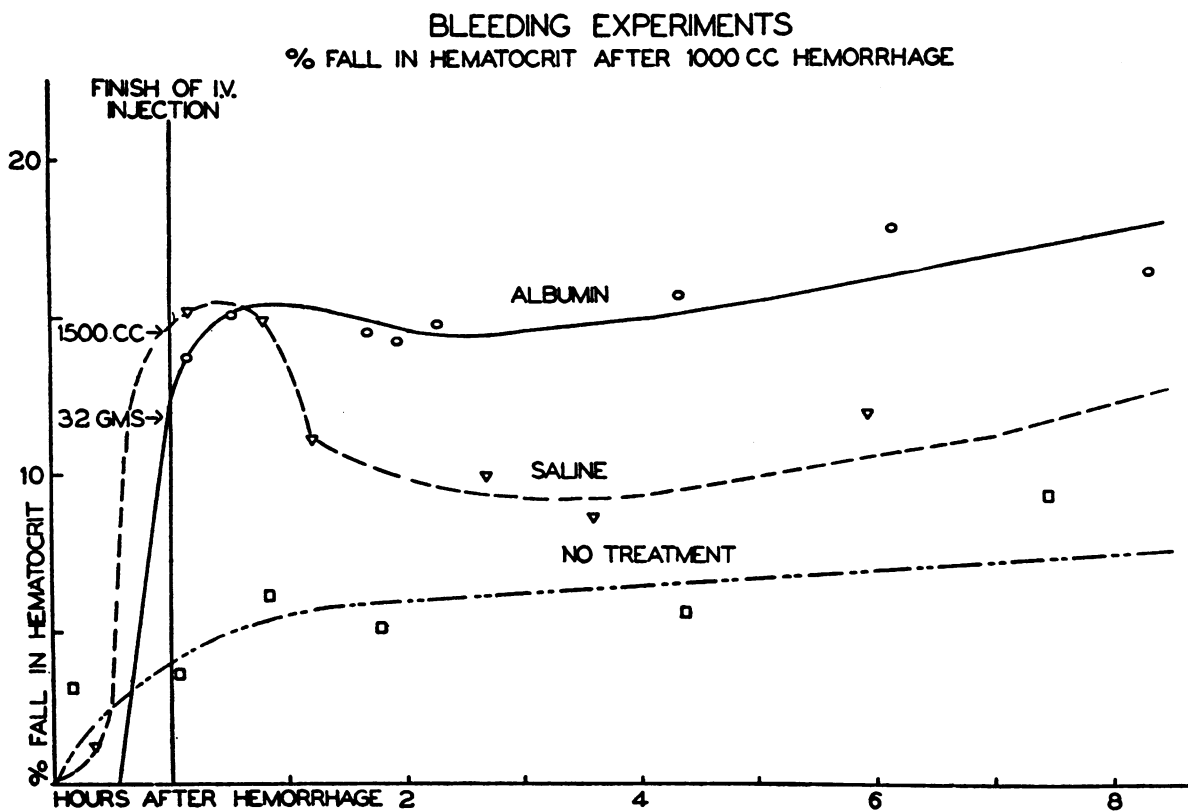


FIG. 2. CHANGE IN HEMATOCRIT READING OF HUMAN SUBJECTS AFTER ACUTE BLOOD LOSS

Note sustained effect of albumin contrasted with transient effect of saline. From data of Ebert, Stead, and Gibson (29) and Heyl, Gibson, and Janeway (5).

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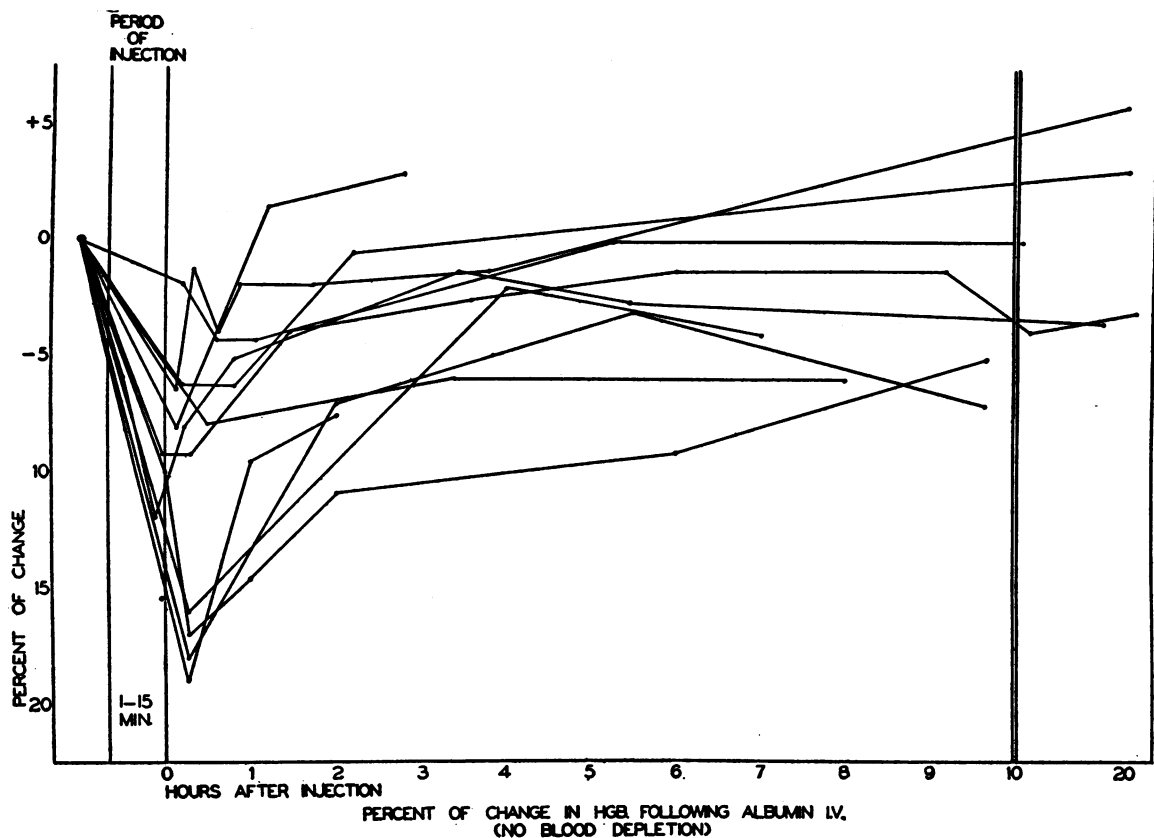


FIG. 3. CHANGE IN HEMOGLOBIN CONCENTRATION AFTER INJECTION OF 25 GRAM DOSES OF CONCENTRATED HUMAN ALBUMIN IN SUBJECTS WITH NORMAL BLOOD VOLUMES

Figures 3 and 4, and is in agreement with the results obtained by Sharpey-Schafer and Wallace with serum (6).

B. Clinical evaluation

The experimental evidence cited above seemed to confirm so well the anticipated theoretical advantages of concentrated albumin that a clinical program was instituted to determine whether the value of albumin could be proved in a series of actual cases of shock. To get a rapid, impartial evaluation, the cooperation of a number of clinics⁸ throughout the United States

⁸ Atlanta, Ga., Grady Hospital; Baltimore, Md., Johns Hopkins Hospital; Boston, Mass., Beth Israel Hospital, Boston City Hospital, Massachusetts General Hospital, Peter Bent Brigham Hospital; Chicago, Ill., Michael Reese Hospital; Iowa City, Iowa, University Hospital; New York, N. Y., Memorial Hospital, Presbyterian Hospital; Philadelphia, Pa., University Hospital; Washington, D. C., United States Naval Hospital, Walter Reed Hospital.

was obtained, and their results were submitted to us for study. At the outset, it was felt that 1000 cases of shock of various types should be treated for the proper evaluation of this material. However, when the first 200 patients had been treated, of whom only 75 were suffering from shock and 25 from burns, and the results analyzed and reported to the appraisal committee, it was their opinion that the value of albumin had been demonstrated, so that no further delay was warranted before recommending it to the Army and Navy. It was also felt that their needs were so urgent that no more albumin should be used for experimental purposes. However, small amounts of albumin subsequently have been allocated to two groups of investigators, whose detailed studies of selected shock cases might contribute information of value to the armed forces. The results of these investigations are presented in subsequent papers in this series (7, 8). The results in the original

group of 200 cases have been previously reported (9).

1. *Methods of study*

The clinical program was set up in an attempt to test the effect of varying doses of albumin in the treatment of different types of shock and to collect all data possible on the advantages and disadvantages of this material. Emphasis was placed upon attempting to demonstrate an increase in circulating blood volume, such as had been observed in experimental subjects. Since it was not feasible to determine the plasma volume in these cases, it was decided to rely upon changes in the hematocrit reading as an index of changes in the plasma volume.

Case reports were made on special forms sent to all cooperating physicians. The data on these were carefully studied in preparing a report for the appraisal committee. That report (9) and the present paper are based on this analysis of cases, treated in cooperating clinics, and on personal observation of most of the cases treated in Boston hospitals, where two of us were on call at all times and carried on the evaluation under the helpful guidance of Dr. Soma Weiss.

Because of the small number of cases, a statistical analysis would be of little significance, particularly in view of the variation in types of cases. Reliance could not be placed entirely on laboratory data, because it was necessarily inadequate in many instances. In cases which showed marked clinical improvement without change in

the recorded hematocrits, the results were considered satisfactory, particularly in those patients who received saline before treatment with albumin was instituted. In some instances, the physician did not withhold plasma or blood indefinitely. Such cases often proved particularly instructive because of this other treatment as, for example, those showing little or only temporary response to saline, but marked and sustained improvement with albumin. Likewise, cases showing little response to albumin and later no further response to whole blood transfusion, made a more accurate interpretation of the results possible. The administration of additional fluids prevented an evaluation of the effects of albumin in dehydrated patients, but since all the traumatic shock cases were treated soon after injury, none was sufficiently dehydrated to have provided information on this point.

2. *Results*

The cases of shock have been classified into several groups: traumatic, hemorrhagic, operative, and shock associated with infection. Cases of traumatic shock often overlapped those of hemorrhagic shock, and such cases were classified according to which seemed to be the more important factor. Each group of cases was analyzed with respect to the number showing improvement in general condition, hematocrit

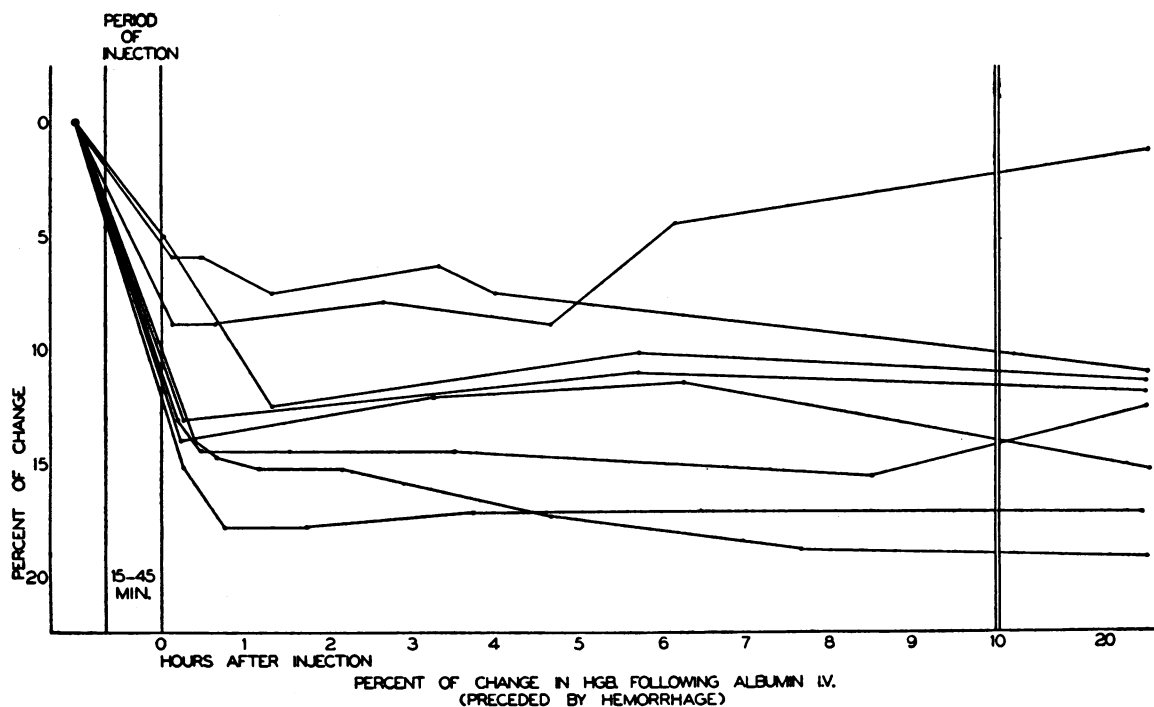


FIG. 4. CHANGES IN HEMOGLOBIN CONCENTRATION AFTER INJECTION OF VARYING DOSES OF CONCENTRATED HUMAN SERUM ALBUMIN IN SUBJECTS WITH BLOOD VOLUMES DEPLETED BY HEMORRHAGE

TABLE I
Selected shock cases

Diagnoses	Clinical condition		Blood pressure		Pulse		Hematocrit		Other fluids		Remarks	
	Before treatment	After treatment		Before	After	Before	After	Before	After			
		1/2 hour	1 to 2 hours							Before		After
A. TRAUMATIC SHOCK												
Compound fracture, skull and humerus	Mild shock	Much improved	B.P. still elevated	70/40	140/80	120				0	0.25 L. blood 2 to 3 hours later	Prompt restoration of B.P. with improved clinical condition allowing skull repair.
Fractured femur	Mild shock	Definitely improved	Stabilized	80/50	100/60	120	106	23	23	1 L. saline	0	B.P. promptly restored and maintained in contrast to previous saline response which was not maintained. First dose gave prompt improvement. Blood transfusion at 3 hours. Relapse at 8 hours.
Compound fracture, humerus	Severe shock	Improved	Good for 3 hours	Unobtainable	158/60	No pulse	76				10 per cent D in S; blood at 3 hours	Prompt response with 2nd dose. Followed with transfusion. Died 14 hours later.
Same case 8 hours later	Severe shock	Improved	B.P. maintained	Unobtainable	160/50	No pulse	120				0.5 L. blood	Prompt response with 2nd dose. Followed with transfusion. Died 14 hours later.
Fracture, skull, jaw, and humerus	Profound shock	Improved	Died	Unobtainable	54/32	No pulse	145	46	35	0	0	B.P. improved markedly from profound shock. Died of skull injury.
Fracture, humerus, both femurs	Severe shock	Good	Good	90/60	110/72	No pulse	96			Saline	0	Saline brought pressure up to 90/50. Albumin gave additional response in 2 doses and B.P. maintained.
Fracture, skull, both tibiae, fibulae, pelvis	Severe shock	Improved	Improved	88/60	115/70	96	100			0.5 L. saline	1 L. saline	Albumin and saline maintained good B.P. in hopeless case long enough for operative treatment.
Compound fracture, both tibiae and fibulae	Severe shock	Fair	Good	80/40	90/45	130	72	40	29	0	1 L. saline	Condition improved and sustained with albumin and saline.
Fracture skull; contusion of brain	Grave	Much improved	B.P. 94/50 before blood	70/40	86/50	144	168			0	5 per cent D in S	Appeared much improved with albumin and saline. Later received 50 per cent D and blood.
Contusion of abdomen	Severe shock	Warm, dry good pulse	Good	?	140/90	Feeble 70	Strong 70	62	48	0	0.5 L. blood post-op.	Striking improvement with albumin alone. At operation, much serous fluid found in abdomen.
Compound fracture femur, multiple lacerations	Mild shock	Good	Satisfactory for 2 hours	90/60	90/60	120	120	36		1 L. D in S	0	Relapsed 24 hours later. See below.
Same case 2 1/2 hours later	Moderately severe	Good	Good	60/20	110/60	145	145			0	0	Later transfused because of tachycardia and low hematocrit.
B. HEMORRHAGIC SHOCK												
Bleeding esophageal varices	Shock, cold, wet	Not in shock	B.P. 70/48	80/55	100/76	72	76	8	7.5	0.1 L. saline	Saline continued	Albumin injected into saline tube. B.P. rose in 4 minutes.
Laceration of neck and wrist	Severe shock	Improved	B.P. 115/70	60/?	110/80	120				0	1 L. D in S	Rapid and marked clinical improvement.
Bleeding from splenic artery	Severe; no B.P. for 45 minutes	Fair	Good	Unobtainable	110/60	180	120	45	41	1.25 L. blood 20 cc. extractin	0.75 L. blood	Patient was not responding to transfusion and exitus considered probable. Albumin response dramatic.
Gastrointestinal hemorrhage	Severe shock	Improved	Improved	70/48	90/40	140 weak	118	19.7	14.7	0	2 hours later 0.52 L. blood	Apparently maintained status quo despite continued hemorrhage.
Incomplete abortion	Mild shock	Improved	Improved	80/0	90/60	140	100			0	0.5 L. saline after 45 minutes	Clinical condition showed marked improvement after albumin.
Bleeding duodenal ulcer	Severe shock	Improved	Improved	50/38	112/54	150	100	16.5	14.1	0.5 L. D in S	4 hours later 0.5 L. blood	Immediate response of pulse and blood pressure to albumin.

reading, and blood pressure, and then an attempt was made to evaluate the results in each case, as influenced by the severity and type of injury and by other forms of treatment. A summary of the data in some of the cases of traumatic and hemorrhagic shock is given in Table I.

Eighteen of the cases were in severe or profound shock as a result of very extensive injuries. With one exception, these cases were suffering from fractures, the majority being multiple and 14 of them compounded. Seven had fractures of the skull, 3 others having brain injuries. The reason for the selection of such severe cases was that albumin was usually not requested if the physician treating the case felt that saline alone would suffice.

Twenty-five cases of traumatic shock were treated, among whom the clinical effect was interpreted as good in 17 and fair in 3. The 5 showing no clinical change were all hopeless cases, and in 3 of these, transfusions were of no avail, while in 2 others, the blood pressure and pulse were definitely improved after albumin. Six patients died from the severity of their injuries but showed good response to albumin for a period and would almost certainly have died with any form of therapy; indeed, several were transfused without benefit. The blood pressure recorded in 24 cases showed improvement in 19. Of the remaining 5, 3 continued to bleed, while 2 were *in extremis* at the time of treatment. Hematocrit levels before and after treatment were obtained on 11 and showed prompt hemodilution in all except 2. These cases, although in profound shock, received less than half the usual dose.

Little evidence concerning the duration of the effect of albumin could be gained in these early cases as they were so severely shocked and injured that in only 2 instances was other therapy withheld for more than an hour. In these cases, improvement continued. In 2 cases, the blood pressure rose rapidly and further bleeding occurred, which was difficult to control.

In a few instances, albumin was given prophylactically to patients who, the physician believed, would go into shock. None of these developed shock subsequent to the injection.

Sixteen cases in shock from hemorrhage were

treated, with clinical improvement in 13. The response was interpreted as marked or striking in 10. Two who failed to improve had uncontrolled hemorrhage and 1 received less than 12 grams of albumin. Hematocrit readings done on 6 showed a fall in each instance, varying from 5 to 50 per cent.

The operative shock group consisted of 25 cases. With 1 exception, clinical improvement was noted in all who received more than 12 grams. Hematocrit levels were suitable for interpretation in 21 cases and showed hemodilution in all but 1. Three died later because of their disease.

Although transient hemodilution followed the injection of albumin in cases of shock associated with severe infection, the ultimate results were as unsatisfactory as the results obtained by other investigators with blood or plasma (10). In 2 cases of shock from acute abdominal conditions, where there was marked dehydration, the results with concentrated albumin were unsatisfactory until saline solution was administered. Clinical improvement was then noted. That this failure was due to lack of available extravascular fluid was suggested by the fact that although the hematocrit fell somewhat, the serum protein concentration rose more than usual. A fall in both figures occurred following the administration of saline.

Twenty-five cases of burns were treated, 18 of them in the acute phase. Local treatment used in these cases was as follows: tannic acid, 7; triple dye, 3; triethanolamine and sulfadiazine, 3; boric acid ointment and pressure dressings, 1; not reported in others. Several received only concentrated albumin in the first stages, and it was shown that hemoconcentration could be effectively reduced by this treatment. In 1 case, the hemoglobin value of 21 grams per cent was reduced to 16 grams per cent within 1 hour after 25 grams of concentrated albumin. Four hours later the hemoglobin had risen again to 18 grams per cent, and was reduced to 15.4 grams per cent after a second dose. This is probably not ideal treatment, but does demonstrate the effectiveness of concentrated albumin, even in a very severely burned patient (80 per cent of body surface). Many of the patients received albumin as a 5 per cent solu-

tion diluted with physiological saline, and this also was effective in reducing hemoconcentration.

In several cases, the rapid hemoconcentration which follows saline infusion was well illustrated, and the hemoglobin value was brought back to the normal range by the administration of albumin (Figure 5). Since burn patients may require repeated doses over a period of many hours, 1 severely burned patient was treated almost entirely with albumin and saline and glucose solutions for 27 hours in an attempt to determine whether any adverse effects would occur. The burn involved 50 per cent of the body surface, and after debridement, an eschar was formed with tannic acid and silver nitrate. The total dose amounted to 450 grams of albumin (18 passages), and 1 liter of plasma, the equivalent of more than a 2-fold replacement of his total circulating plasma (Figure 5). At the

end of 27 hours, his hemoglobin was within normal limits, and he did not go into secondary shock. However, after the first 300 grams (12 passages) had been administered in a period of 18 hours, oozing of blood was observed about the intravenous cannula and it was found that his serum globulin had fallen to an extremely low level (albumin/globulin ratio of 6.7/0.7) and that the prothrombin time was slightly prolonged. Both returned to normal following the use of whole fresh plasma. This is the only case in which were noted the effects of a deficiency of serum globulin following albumin therapy.

A dose of 25 grams was found to be satisfactory in this group of clinical cases, although, in many instances, a second dose was necessary. A dose of 12.5 grams (the equivalent of 250 cc. of citrated plasma) was not sufficient in the majority of cases where this amount was used,

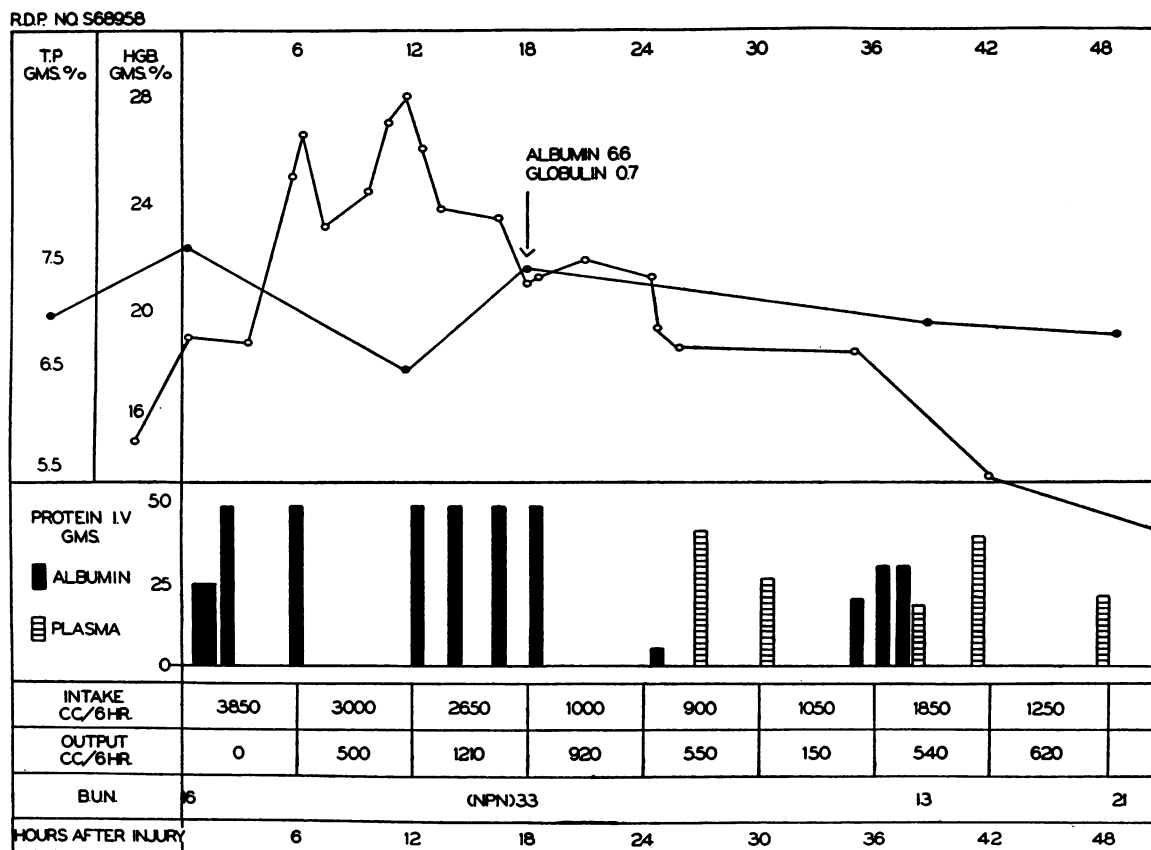


FIG. 5. CLINICAL COURSE OF A SEVERELY BURNED PATIENT (50 PER CENT OF BODY SURFACE) IN THE FIRST 48 HOURS

No fluid was given by mouth during this period. Note rapid hemoconcentration following intensive administration of saline and glucose solutions at 6 and 12 hours and its prompt reduction by albumin injections.

as has been borne out by the decision to increase the size of the Standard Army and Navy dried plasma package to 500 cc. It was our conclusion that the best practice was to infuse 25 grams of albumin rapidly and to repeat this dose about 15 minutes later if a sufficient response had not been obtained. The reason for repeating the injection so promptly was that most of the hemodilution usually occurred within this period (Figures 1 and 4). In burns, there is continued loss of fluid from damaged capillaries, and it is not logical to draw on the extravascular fluid stores for more than emergency treatment. Consequently, we have used albumin diluted to 5 per cent with saline solution (2 packages of albumin per liter of fluid). The amount necessary varied with the extent of the burn and appeared to be comparable to the requirement for plasma.

C. The Standard Army and Navy Package of Serum Albumin

The Standard Army and Navy Package of Serum Albumin was developed in order to provide albumin in compact form, easily available for rapid administration (11, 12). It has been repeatedly demonstrated that this package can be opened and administration started in 1 minute, with less than 10 minutes required for the infusion of its contents by gravity.

The package consists of 100 cc. of a solution containing 25 grams of human serum albumin in 0.3 molar sodium chloride at a pH of approximately 6.8, with merthiolate 1:10,000, or its equivalent, as a preservative. These conditions of solution have been shown in a preceding study (13) to assure adequate thermal stability of albumin. The double-ended glass container with a small vaccine stopper at each end, together with sterile airway needle and complete intravenous equipment, suspension tape, instructions, and questionnaire are sealed in a metal can. The saving of space which concentrated albumin makes possible is best shown by the figures in Table II.

Discussion

If albumin were dispensed as an isosmotic (5.6 per cent) protein solution, few objections could be raised to its use in the treatment of

TABLE II
Package size of osmotically equivalent blood substitutes

	Number of units	Plasma volume osmotically equivalent	Size of package	Weight of package
		cu. cm.	cu. in.	lbs.
<i>Dry plasma</i>				
250 cc. Unit	2	500	480	7.5
500 cc. Unit	1	500	300	5.3
<i>Albumin</i>				
100 cc. Unit	1	500	48*	0.9*

* Taken as one-third of carton containing 3 units.

conditions for which an osmotically active solution is indicated. However, since it is dispensed as a 25 per cent solution, for reasons of military expediency, many may question its use in the treatment of shock. The effectiveness of albumin in augmenting blood volume depends upon its ability to draw fluid from the tissues into the blood stream. When there is sufficient extracellular fluid available, this takes place, as has been demonstrated in the foregoing experimental and clinical studies.

The important question is whether the administration of concentrated albumin to severely dehydrated patients is either ineffective or harmful. The clinical cases shed little light on this, since additional saline solution was administered to the majority of patients and only 5 of the 200 cases were markedly dehydrated. In 2 of these patients only, where there had been vomiting as a result of acute abdominal conditions, was albumin relatively ineffective in producing clinical improvement until additional fluids were given, even though some fall in hematocrit followed its administration. On the basis of the available data, the appraisal committee made the following recommendation, which is stamped on each albumin bottle: "Precaution:— In the presence of dehydration, albumin must be given with or followed by additional fluids." It is not necessary that this fluid be given intravenously if the patient can take water and salt by mouth or retain a proctoclysis.

Subsequently, with crystallized bovine albumin⁹ available for animal experimentation,

⁹ Prepared at the Armour Laboratories by the method of Cohn and Hughes (14).

data have been obtained which substantiate the wisdom of the committee's recommendation. Fine, Frank, and Seligman (15) have reported that in tourniquet shock in dogs, which in their hands was almost uniformly fatal if untreated, concentrated albumin was ineffective. However, if saline were given by stomach tube, 25 per cent albumin was as effective as 5 per cent albumin or plasma. Their protocols do not show that concentrated albumin was harmful *per se*, but in the absence of sufficient extravascular fluid, it failed to produce an adequate increase in plasma volume. Mahoney, Kingsley, and Howland (16) have shown that in the treatment of severe shock due to intestinal trauma in dogs, dilute and normal plasma were more effective than concentrated plasma. Twenty-five per cent albumin did more than concentrated plasma to restore the circulation, but the animals did not survive (17). Dunphy and Gibson (18) compared the effect of concentrated and dilute albumin solutions with homologous plasma in the treatment of severe burns in dogs. Concentrated albumin was effective in sustaining the circulation, but microscopic examination seemed to indicate slightly greater tissue damage in animals receiving the concentrated solution as compared to dogs receiving plasma or dilute albumin. We bled 2 splenectomized dogs, which had been dehydrated by withholding all water for 72 hours, until the blood pressure fell to shock levels, and the animals were stuporous and cold. Large doses of concentrated crystallized bovine albumin were then injected, and the animals responded with a rise in blood pressure, hemodilution, and marked clinical improvement, so that they were able to get up and walk about the room, somewhat unsteadily. The serum protein concentration rose from 6.5 to 8 grams per cent. Four hours after albumin the dogs were offered water and saline solution. They drank both and then became normal in behavior, a fall in serum protein and hematocrit showing that further hemodilution had occurred.

Thus, the experimental data indicate that the effectiveness of concentrated albumin in the treatment of blood and plasma loss may be somewhat limited by the extent to which the patient is dehydrated, although this factor was not important in actual cases of shock due to

trauma or hemorrhage. There is little evidence that albumin is harmful even in the presence of severe dehydration, but the deficiencies of fluid and electrolytes, characteristic of this condition, should be remedied as soon as possible if a maximum therapeutic effect is to be obtained.

A second objection, frequently raised to the use of albumin, is that since it lacks the globulins of the plasma, its administration in large doses may lead to a deficiency of those globulins concerned with blood coagulation and immunity. Earlier studies in dogs and humans receiving bovine albumin showed that there was a considerable margin of safety before a diminution of the antibacterial or phagocytic powers of the blood could be detected (19). In our group of patients, there was nothing to suggest a lack of immune bodies. Relative globulin deficiency, as indicated by a low serum globulin concentration was observed only twice, and in only 1 patient did oozing of blood occur which could be correlated with a prolonged prothrombin time. A comparison of the relative ability of albumin and aged liquid plasma, which contains no functionally active but only denatured globulins, to promote the regeneration of prothrombin and complement should be both interesting and important. In severe burns, plasma rather than albumin is obviously the therapeutic agent of choice, but we have demonstrated in 1 patient at least that the osmotic equivalent of nearly twice the calculated plasma volume can be replaced with albumin and saline solution before serious signs of globulin deficiency appear.

It should be emphasized that neither albumin nor plasma supply red cells, with their capacity for transporting oxygen. Thus, the continued use of protein solutions, to replace losses of both plasma and blood in patients with war injuries, will inevitably result in the development of anemia, unless red cells are given to make up this deficit.

Since a small volume of albumin injected intravenously induces a considerably greater increase in the volume of the circulation, some caution should be used in its administration to patients with a low cardiac reserve. We have observed a few cases of shock in whom bleeding had not been controlled before treatment, who lost

considerably more blood when the blood pressure rose rapidly after albumin injection. This is a principle of hemodynamics which applies to any effective form of therapy.

Summary of Part I

1. Concentrated human serum albumin increases blood volume rapidly by drawing extravascular fluid into the circulation.

2. The hemodilution which follows albumin injection is well sustained in patients with previously depleted blood volumes, but is transient in those with normal blood volumes.

3. As would be expected from *in vitro* measurements, approximately 18 cc. of fluid is added to the blood stream for each gram of albumin injected after hemorrhage, with wide variation in individual instances. Thus, the standard package of 25 grams of albumin in 100 cc. of diluent is equivalent in its osmotic effect to 500 cc. of citrated plasma.

4. Prompt hemodilution and clinical improvement have been shown to follow the injection of concentrated albumin in a group of cases of shock and burns.

5. Clinical and experimental data indicate that concentrated albumin is not harmful in cases of shock with severe dehydration, but is more effective if water and salt are also administered by any available route.

6. Clinical effects of a deficiency of the serum globulins have been noted in only 1 case—a severely burned patient, who had received 300 grams (12 passages) of albumin in 18 hours.

7. The Standard Army and Navy Package of Normal Serum Albumin (Human) concentrated, provides a stable blood derivative in compact form, instantly available for rapid administration, which has been developed to meet the needs of highly mobile military units.

PART. II. SAFETY OF ALBUMIN

As commercial production of albumin for the armed forces began, a rigid system of control was considered necessary. Therefore, after each lot of albumin had undergone physicochemical as well as the usual sterility, animal safety, and rabbit pyrogen tests, samples were used clinically before it was released.

A. Methods of study

Since more than 1900 such intravenous injections of albumin have been given in the past 20 months, it is evident that it was not feasible to test each sample on a separate subject. Accordingly it was decided to give multiple injections whenever possible to patients with hypoproteinemia, who might be expected to benefit from albumin therapy. In addition to a diminished serum protein level, the cases had to have a relatively normal temperature. Those with cardiac disease or allergy were excluded. Patients selected according to the above criteria were given the test lots of albumin by gravity, using the intravenous kit included in the package. The temperature and pulse were recorded before injection and each hour thereafter for 3 to 4 hours, the patients being carefully observed during this period.¹⁰ The albumin was administered as the standard 25 per cent solution in 0.15 or 0.3 molar sodium chloride, buffered with sodium bicarbonate to a pH of from 6.6 to 7.0, and containing 1:10,000 merthiolate, or its equivalent, as a preservative. A dose of 20 to 25 grams was given at each injection.

B. Results

1. Amounts given

From April 1942 (when the first standard albumin prepared for the armed forces was tested) to December 1943, 1915 injections of albumin were given to 600 patients. This included 250 injections of material heated for varying periods at different temperatures before administration. Forty-four patients received more than 10 injections. Five patients received over 800 grams each, 3 of these receiving over 1000 grams. The 3 longest periods of treatment extended over 69, 342, and 570 days. Six patients received an average of 25 or more grams of albumin daily for from 5 to 20 days.

2. Reactions

In our hands, properly processed albumin has been administered routinely without reaction. However, in the use of albumin submitted for approval, particularly in the early days of commercial production, we encountered a certain

¹⁰ We are indebted to the members of the visiting, house, and nursing staffs of the following hospitals for their cooperation: Beth Israel, Children's and Infants', Boston City, Boston Floating, Massachusetts General, Massachusetts Memorial, and Peter Bent Brigham Hospitals, Boston, Mass.; U. S. Naval Hospitals in Chelsea, Mass. and Bethesda, Md.; Doctor's, Gallinger Memorial, Garfield, Providence, and Sibley Hospitals, Washington, D. C.

number of pyrogenic reactions. Such lots were rejected until the albumin had been reprocessed and repackaged in a satisfactory state. Data are not available on the incidence of reactions in routine use of the lots which have been released, but since no preparation which gave reactions on test has been accepted, it should be extremely low.

In all, seventy-seven pyrogenic reactions occurred (in the course of these studies) in 56 patients given 39 lots. Fifty-five reactions consisted of chills and fever, while in 22, fever was the only manifestation. The average recorded temperature rise was about 2° F. The interval before onset of chills was usually under 1 hour with an average duration of about 30 minutes. No anaphylactoid symptoms were observed, following single or repeated injections of standard lots.

Cardiovascular symptoms due to rapid increase in the volume of the circulation appeared in some, such as increased pulse and blood pressure, but no pulmonary edema was seen.¹¹ Severe pyrogenic reactions in 3 patients were followed by hematemesis from esophageal varices. One patient with nephrosis died in convulsions the day after a severe pyrogenic reaction, which was presumably due to bacterial contamination of a single bottle, since intensive study of the rest of this lot showed that it was satisfactory. Three patients died from pulmonary embolism within 24 hours of albumin injection.

Since albumin was developed for use in places where refrigeration is not available and the weather apt to be hot, it was important to demonstrate that material which had been exposed to a high temperature did not produce reactions. Actually, the incidence of pyrogenic reactions was reduced by exposure of albumin to heat. Some heated samples showed visible changes, consisting of a darker color, slight turbidity, and fine floccules. Many of these have been administered without difficulty, but to assure safety, a fine wire mesh filter has been incorporated in the intravenous equipment of the standard package. In the group of 250 pa-

¹¹ One patient developed pulmonary edema following repeated albumin injections in the early days of clinical testing, before the methods for the preparation of albumin had become standardized.

tients given heated albumin, no anaphylactoid reactions occurred. One hundred and thirty-three of these patients (Table III), as well as 72

TABLE III
Injections of heated albumin

Temp. at which heated	Days heated	No. injections	No. followed	Reactions	
				Early	Late
°C.					
37	77 to 134	3	2	0	0
45	31 to 60	2	0	0	
50	1 to 10	15	0	0	
50	12	218	131	6*	0
50	14 to 33	6	0	0	
50	100 to 117	6	0	0	
	Totals	250	133	6*	0

* Pyrogenic reactions in 2 patients with each of 3 unsatisfactory lots.

who received injections of unheated albumin, were followed for from 3 weeks to one year, and no delayed reactions of any kind were observed. No cases of homologous serum jaundice have been discovered (20).

3. Pathologic studies

While the evaluation of human serum albumin chiefly involved clinical and physiologic technics, it seemed important to determine whether or not pathologic changes were produced by the material. Since the use of experimental animals would have introduced the complicating factors of species differences, it was necessary to depend upon human necropsy material for pathologic data. It was essential to attempt the separation of any pathologic changes due to the human serum albumin itself from those due to the preservative (merthiolate) and those due to the disease from which the patient was suffering. While minute histologic alterations resulting from the administration of the albumin might well be masked in such material, tissue changes of importance would be detected.

Several hospitals¹² contributed material for

¹² We wish to express our gratitude to those hospitals which submitted material to us: Peter Bent Brigham, Massachusetts General, Children's, and Boston City Hospitals, Boston; Johns Hopkins Hospital, Baltimore; and New York and Presbyterian Hospitals, New York.

this study. There was necropsy material from 16 patients who received from 13 to 813 grams of human serum albumin. Included in the series were patients dying within a few hours after a single injection, as well as those receiving large quantities in repeated injections over several months.

The results of the examination of tissues from these 16 patients may be summarized briefly. In no organ was there evidence of histologic change which could be correlated with the administration of albumin. Particular search was made for "storage disease," which was not found. Pathologic storage of this material would not be expected in view of the fact that it is native to the human organism and is an integral part of the blood plasma. However, these studies offer objective proof that pathologic accumulation does not take place, even when as much as 813 grams are administered. There was no evidence of periarteritis nodosa in any instance. No renal glomerular damage was encountered which could not be explained by the disease of which the patient died. It was in the evaluation of minute changes in renal morphology that the shortcomings of routine necropsy material were most evident.

One patient (C. B.) with intestinal obstruction and bronchiogenic carcinoma, receiving 650 grams of human serum albumin in 6 days, deserves special comment. The albumin was administered in 25 per cent solution containing 1:10,000 merthiolate, a total of approximately 0.13 gram of mercury. In addition, the patient received 2 doses of salyrgan, containing a total of 0.08 gram of mercury, the last on the day of death. There was considerable swelling, degeneration, and desquamation of the cells of the convoluted tubules of the kidney. In another patient receiving 813 grams of albumin over a period of 2 months, there was less evidence of damage to the renal tubules. The evaluation of these changes was particularly difficult because of the numerous pathologic alterations due to the diseases for which the albumin was given. It seems likely that they were due to the mercury rather than to the human serum albumin *per se*. However, the changes were very minor and could not be interpreted as affecting the clinical course of the patient.

Discussion

The occurrence of pyrogenic reactions in a few lots which had passed the rabbit pyrogen test required by the National Institute of Health justifies the procedure of clinical testing, and since these lots were rejected, the reaction rate with albumin released for distribution should be very low. Most pyrogenic reactions have been mild but have had serious consequences in a few instances. Hematemesis from esophageal varices after a severe chill and pulmonary embolism following a sudden increase in blood volume are not unlike accidents which have been observed after blood or plasma transfusions in any large group of patients with serious chronic illnesses.

The fact that a few patients have received total doses of over 1000 grams, that others have tolerated repeated injections at varying intervals, and that heated albumin has been injected repeatedly without the occurrence of anaphylactoid symptoms, indicates that standard preparations of concentrated human albumin are not antigenic in man, nor do they become so on heating at 50° C.

Summary of Part II

1. In clinical trials of individual preparations of concentrated human serum albumin, 1915 injections were given to 600 patients.
2. Pyrogenic reactions occurred with unsatisfactorily processed material, which was rejected. The reaction rate from albumin lots released for distribution under the rigid system of control used should be extremely low.
3. In 7 of a group of 600 patients, most of whom were suffering from serious chronic illnesses, untoward incidents occurred within a short time after albumin injection. These are discussed in the text.
4. Repeated injections of normal or heated (50° C.) albumin failed to produce any other type of immediate or delayed reaction.
5. Five patients received a total of more than 800 grams of albumin each, 6 patients received 25 or more grams per day for from 5 to 20 days, and 44 patients received more than 10 injections without harmful effects.
6. Pathologic studies of necropsy material from patients who had received albumin failed

to reveal significant changes which could be attributed to albumin.

PART III. ALBUMIN IN THE TREATMENT OF HYPOPROTEINEMIA

In the course of performing the large number of clinical tests described in Part II, an attempt was made to learn as much as possible about the usefulness of concentrated human serum albumin in the treatment of hypoproteinemia. The necessity for expediting the delivery of albumin to the armed forces by testing samples as fast as they were submitted made it difficult to plan or carry out long-range studies along these lines. Consequently, the data are somewhat fragmentary.

A. Methods of study

Cases with hypoproteinemia were selected for study on the basis of the criteria previously mentioned. Patients in a number of Boston and Washington hospitals were treated.

The laboratory determinations were almost all made in our own laboratory. Venous blood was drawn without stasis and mixed with balanced potassium-ammonium oxalate for determination of hemoglobin and hematocrit, while serum was used for chemical determinations. Oxy-hemoglobin determinations were made with the Klett-Summerson photoelectric colorimeter. Plasma volume measurements were made by the method of Gibson and Evans (21) using Gibson and Evelyn's adaptation of the method to the photoelectric colorimeter (22). All nitrogen determinations were made by Kjeldahl digestion and direct nesslerization, using the Klett-Summerson photoelectric colorimeter. Similar methods were used for these determinations in both serum and urine, trichloroacetic acid being used to precipitate the proteins. Serum albumin was roughly estimated, when Tiselius analysis was not considered necessary, by salting out the globulins with sodium sulfate by the Howe method (23), with Kjeldahl digestion and nesslerization of the filtrate. Chlorides were determined in serum and in urine by the method of Schales and Schales (24).

In nitrogen balance studies, records of the actual protein intake were kept by trained dietitians who recorded the amounts offered and those refused by the patient and made the calculations of protein intake. For an analysis of the fate of injected albumin, reliance was placed upon determinations of the output of total nitrogen and total non-protein nitrogen in the urine over stated periods. The urine for each 24-hour period was collected under toluol and kept cold. The urine for a period of 1 to 4 days was pooled and determinations were made from the pooled sample to give an average daily output figure for the experimental period. Determinations of fecal nitrogen output were not made, but were assumed to be relatively constant, 2 grams per day being added to the urinary

nitrogen figure to give the total daily nitrogen excretion. A nitrogen factor for protein of 6.25 was used in all calculations.

B. Results

1. Fate of injected albumin

The transient nature of the hemodilution which follows the injection of albumin into patients with normal blood volumes (Figure 3) suggests that albumin is readily withdrawn from the circulation. Further evidence is provided by the fact that daily doses of 25 grams or more may be administered to hypoproteinemic patients for considerable periods without the appearance of venous congestion. An excellent example of this is presented in Table IV. This

TABLE IV

Response of patient M. P. with acute hepatitis to albumin

Date	Albumin dose	Serum albumin	Serum globulin	Hematocrit
	<i>grams</i>	<i>grams per cent</i>		<i>per cent</i>
January 22, 1943	50	2.2	3.1	24
January 23, 1943	50	2.9	2.5	
January 24, 1943	50	2.6	2.6	23
January 25, 1943	50	3.1	2.1	23
January 26, 1943	0	3.1	2.5	
January 27, 1943	0	3.0	2.7	

patient (M. P.) received 50 grams daily for 4 days without any appreciable change in hematocrit, and with an increase in serum albumin, which accounted for only a small part of the total amount injected.

Tests of the urine for protein were negative after albumin injection in most cases. However, in some patients, definite but negligible quantities of protein were excreted, while in those with the nephrotic syndrome, a considerable portion of the injected albumin could be accounted for by an increase in proteinuria. That portion of the injected albumin which cannot be accounted for in the urine must be metabolized or stored. In the former case, it should appear as non-protein nitrogen in the urine, and in the latter case, it should contribute toward a positive nitrogen balance.

The nitrogen balance has been studied before, during, and after repeated doses of albumin in 3 patients, 1 in the nephrotic stage of chronic glomerulonephritis, 1 with hypoproteinemia com-

plicating constrictive pericarditis, and 1 with cirrhosis of the liver. The data are summarized in Table V, while the results in the patient with cirrhosis of the liver are also presented in Figure 6, since the experiment was technically most

satisfactory in her case. All of these patients had low levels of serum albumin. In each of them, more nitrogen was retained during and immediately after the period of albumin injection than in the control periods. In patients I

TABLE V

Nitrogen balance

(All figures represent average daily value in grams of N for period)

Number days	Experimental period	Oral nitrogen intake	Injected nitrogen	Total nitrogen intake	Urinary non-protein nitrogen	Urinary protein nitrogen	Total† nitrogen output	Balance
I. B.B. NEPHROTIC STAGE OF GLOMERULAR NEPHRITIS								
3	Control	11.6		11.6	7.5	2.2	11.7	-0.1
3	2500 cc. plasma	16.9	8.3	25.1	14.2	5.7	19.9	5.2
1	Control	15.8		15.8	10.1	5.5	17.6	-1.8
4	75 grams albumin	16.4	3.0	19.4	7.2	5.7	14.9	4.5
4	Control	17.1		17.1	7.8	4.9	14.7	2.4
6	Control	16.3		16.3	7.6	4.1	13.7	2.6
4	100 grams albumin	17.7	4.0	21.7	9.3	5.9	17.2	4.5
3	Control	17.0		17.0	6.0	3.4	11.4	5.6
8	Control	16.4		16.4	8.5	3.9	14.4	2.0
8	Control	15.8		15.8	10.6	3.6	16.2	-0.4
II. F.G. CONSTRICTIVE PERICARDITIS								
18	Control; high protein diet.	15.7		15.7	11.8	0.0	13.8	1.9
4	Control; low protein diet.	8.1		8.1	5.2	0.0	7.2	0.9
4	87 grams albumin; low protein diet.	8.9	3.4	12.3	4.8	0.0	6.8	5.5
6	125 grams albumin; high protein diet.	17.7	3.3	21.0	13.4	0.0	15.4	5.6
4	Control	17.4		17.4	11.8	0.0	13.8	3.6
14	300 grams albumin	16.0	3.4	19.4	12.6	0.0	14.6	4.8
6	Control	16.5		16.5	12.0	0.0	14.0	2.5
III. A.P. CIRRHOSIS OF LIVER								
3	Control	10.1		10.1	7.5	0.0	(9.5)* 10.3	(0.6)* -0.2
3	Control	10.5		10.5	7.2	0.0	(9.2)* 10.0	(1.3)* 0.5
5	Albumin	10.2	4.0	14.2	6.3	0.9	9.2	5.0
4	Albumin	10.8	4.0	14.8	6.0	0.3	8.3	6.5
5	Albumin	11.2	4.0	15.2	7.0	1.2	10.2	5.0
3	Control	10.7		10.7	7.5	0.0	9.5	1.2

* Figures in parentheses indicate N output and balance excluding paracentesis, which removed 5200 cc. of ascitic fluid which reaccumulated during control periods.

† Total urinary nitrogen plus 2 grams (estimated) of fecal nitrogen.

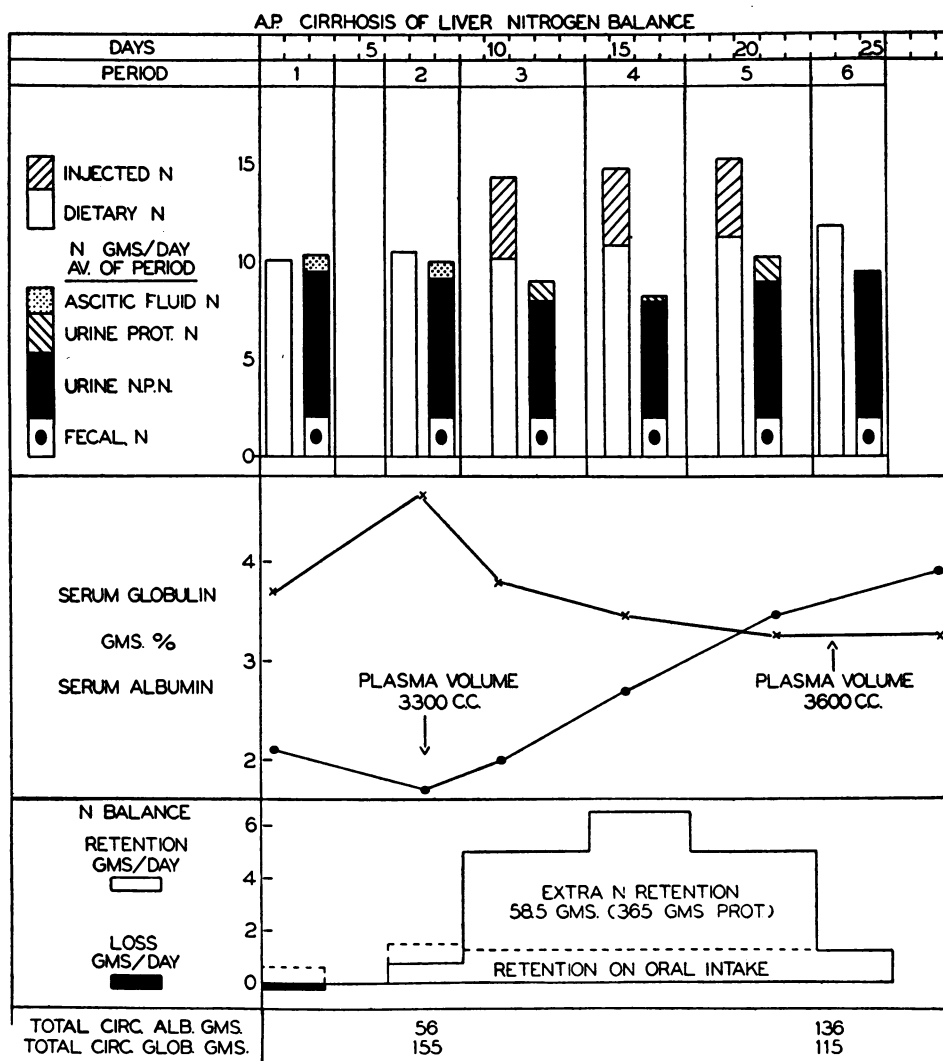


FIG. 6. NITROGEN BALANCE STUDIES IN A PATIENT (A. P.) WITH CIRRHOSIS OF THE LIVER WHO RECEIVED 350 GRAMS OF ALBUMIN IN PERIODS 3, 4, AND 5

and II, interpretation was complicated by variations in the daily protein intake. In patient I, with the nephrotic syndrome, increased proteinuria accounted for a portion of the injected albumin. In patient III, it will be noted that the extra nitrogen retained during the period of albumin injection corresponded very closely with the extra nitrogen injected in the form of albumin. Although the full 350 grams of protein injected were retained, only about 80 grams or one-fourth of it could be accounted for by the increase in total circulating albumin.

Thus, albumin is presumably stored in hypoproteinemic patients, since there is no appreci-

able increase in nitrogen excretion, and it does not appear in the urine unless there is a tendency to proteinuria. This is consistent with the behavior of native plasma protein, and suggests that the albumin molecule has not been significantly altered by the process of fractionation.

2. Results in the treatment of hypoproteinemia

Because of the large amounts of protein necessary to achieve a therapeutic result in hypoproteinemia, only a few patients can be said to have received adequate doses. These patients may be divided into several groups, according to the major cause of hypoproteinemia; (a) those

with an inadequate protein intake; (b) those with altered ability to synthesize serum albumin; (c) those with excessive loss of protein from the body; and (d) a group of miscellaneous cases.

(a) *Hypoproteinemia due to inadequate protein intake.*

Cases

(1) E. M., an emaciated woman in her seventies, entered the hospital because of weakness, dyspnea, and ankle edema. She had been living on a meager allowance, very little of which was spent on food. On admission, attention was focussed on her heart because of paroxysmal auricular fibrillation, râles, pleural effusion, and ankle edema. Despite digitalis and rest in bed, her general condition improved little in the first 3 weeks, and, on a ward diet with vitamin supplements, the serum protein value fell from 5.6 to 5.1 grams per cent. At this point, without change in her diet, treatment with albumin was begun in doses of 20 to 25 grams nearly every day. The results were striking; strength, alertness, and appetite improved. No cardiac embarrassment occurred. Her edema, which had partially subsided before the institution of albumin treatment, disappeared completely, and she left the hospital markedly improved 66 days after admission. The total dose of albumin administered in 40 days to this woman weighing only 84 lbs. was 660 grams. The rise in serum albumin, shown in Table VI, accounted for only a small portion of that administered.

TABLE VI

*Changes in serum proteins of patient E. M.
with chronic malnutrition*

Date	Albumin injected	Serum albumin	Serum globulin	Total protein
	<i>grams</i>	<i>grams per cent</i>		
June 15, 1943	0.0			5.6
July 4, 1943	0.0	2.8	2.3	5.1
July 16, 1943	180.0	3.9	3.0	6.9
August 11, 1943	600.0	5.0	3.0	8.0

(2) W. S. was a 71-year-old man who developed symptoms of intestinal obstruction, proved at necropsy to be due to stricture of the ileum. His serum protein level was extremely low (albumin 1.1 per cent, globulin 2.3 per cent), and therefore, after Wangensteen suction was instituted, he was given daily injections of albumin for 11 days. Because of difficulty with veins from massive edema, this was administered intrasternally through a needle which was left in place between injections. The albumin flowed in readily for the first 3 days, but ran more slowly thereafter, and none could be administered by this route after a week. He took only a small amount of nourishment by mouth and received daily infusions of glucose and albumin. After 320 grams had been given over an 11-day period, there was some improvement of his edema and his serum proteins had risen (albumin 2.0 per cent, globulin 2.0 per

cent), but he failed to gain sufficient strength for operative treatment and died.

(3) C. B., mentioned in Part II, had severe hypoproteinemia following an operation for intestinal obstruction. Large amounts of albumin were available and he was given as much as 125 grams per day, a total of 650 grams in 6 days. This raised the serum albumin level from 2.4 grams per cent to a final value of 5.2, with a serum globulin level of 0.7 per cent. There was marked reduction in edema and clinical improvement at first, but signs of pulmonary congestion subsequently appeared and the patient died in pulmonary edema following an injection of salyrgan the day after his last dose of albumin. The post-mortem findings included generalized edema, carcinoma of the lung, and minor changes in the renal tubules which were attributed to mercury.

From these 3 patients, it was concluded that the serum albumin level can be raised by the administration of large doses of albumin to severely depleted patients. While the second patient did not receive sufficient albumin to relieve his deficiency, it seems probable in the third case that the pulmonary congestion and edema were at least partly due to excessive doses of albumin. Thus, two limiting factors to the speed with which the serum albumin level can be brought back to normal appear to be (1) the capacity of the circulation to adjust to rapid increases in plasma volume, and (2) the rate at which the tissues can metabolize or store excess albumin.

(b) *Patients with altered synthesis of serum albumin.* Cases of portal cirrhosis with low serum albumin levels were treated because they are frequently unable to maintain an adequate level of serum albumin, even on a high protein diet (25). Most of the patients in this group had ascites, and all had had repeated paracenteses. Although it was felt that the ascites was due to at least two factors, increased venous pressure in the portal system, and low total colloid osmotic pressure of the blood, it was hoped that if the serum albumin level could be raised sufficiently, the rate of formation of ascitic fluid might be diminished.

Cases

(1) A. P. was a 65-year-old woman whose condition had been diagnosed as portal cirrhosis, 14 months previously. At that time, 3 paracenteses were required in 2 months. Abdominal fluid then accumulated slowly until 1 year later, when 25 liters were removed in 5 paracenteses, the last 2 of which were performed after admission to the

hospital. At this time, she was in a good state of nutrition and complained only of the discomfort from her protuberant abdomen. Her spleen was not enlarged, and no esophageal varices were demonstrated.

She was placed on a 65-gram protein diet and 25 grams of albumin were given daily for 14 days, during which time she lost a slight amount of weight. Her serum albumin level increased from 1.7 to 3.9 grams per cent during this period, whereas her serum globulin decreased from 4.7 to 3.8 grams per cent. Nitrogen balance studies (Figure 6 and Table V) showed complete retention of the injected albumin. Electrophoretic patterns of her serum before and after treatment (Figure 7) clearly indicated the increase

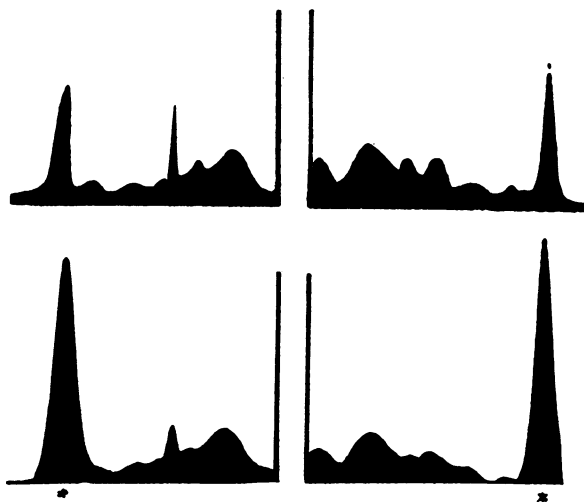


FIG. 7. ELECTROPHORETIC SCHLIEREN DIAGRAMS OF THE SERUM OF PATIENT A. P. WITH CIRRHOSIS OF THE LIVER BEFORE (UPPER) AND AFTER (LOWER) TREATMENT WITH 350 GRAMS OF ALBUMIN

Note increase in albumin fraction (*).

in albumin. A bromsulfalein test on admission showed 60 per cent retention in 30 minutes, and this was recorded as 30 per cent following albumin therapy.

She was then followed at intervals in the clinic, her weight remaining essentially constant during the next 6 months despite the fact that the serum albumin had fallen, during the 2 months after discharge, to 2.5 grams per cent. It remained at this level during the next 4 months without increase in abdominal fluid. When seen 10 months after discharge, her serum albumin had fallen to 2.1 grams per cent and during the preceding months, she had noted a distressing increase in ascitic fluid. At this time, it was felt she was entering the terminal stages of hepatic insufficiency, and she required 5 more paracenteses in the next 2 months.

It was concluded from this case that the serum albumin level in cirrhosis could be elevated if sufficiently large doses were given, but that only a portion of the injected protein would be re-

tained in the circulation. The increase in serum albumin was lost in a period of 2 months, and the interval between series of paracenteses did not seem appreciably affected.

(2) M. C., another patient with portal cirrhosis (Figure 8) entered the hospital, bed-ridden and emaciated. His abdomen was very distended, and a large spleen and esophageal varices were found. A series of 3 paracenteses removed about 20 liters of fluid. At this time, the serum albumin was 1.6 grams per cent and the serum globulin 6.2. For 10 days, the patient was given daily doses of 25 grams of albumin, and at the end of this period, 3.8 liters of fluid were removed from his abdomen. The serum albumin level had risen to 3.5 grams per cent at this time and the globulin had fallen to 4.1. During the next 7 weeks, he received 34 more injections of albumin with but slight further change in his serum proteins and a steady decline of his hemoglobin concentration. His weight increased slowly, levelling off when the abdomen became tightly distended. Fourteen liters of fluid were removed in the 2 weeks following treatment, and at the end of this time, the serum albumin level had already decreased to 2.3 grams per cent. Despite this, he did not require removal of fluid for 6 weeks, a period quite comparable to that while in the hospital. The one striking change noted in this patient was the improvement in general condition during treatment. Although bed-ridden, emaciated, and somewhat delirious on admission, he became stronger, his color and appearance improved, and by the time of discharge he was up and about. Three months later, when seen for the last time, his general condition again approached that at the time of admission, and death occurred a few weeks later.

This patient received very large amounts of albumin (950 grams) during a period of 8 weeks, but in spite of the fact that the serum albumin rose quite satisfactorily within the first 2 weeks, abdominal fluid continued to accumulate at nearly the same rate, and when treatment was omitted the albumin level in the blood dropped soon afterwards. Only the improvement in his general state of nutrition seemed noteworthy.

Four other cases of portal cirrhosis, all of them advanced, were treated and confirmed the previous findings. The serum albumin level was raised to some extent in each case, usually with a slight fall in serum globulin, and in several, there was a distinct improvement in nutrition. The evidence is not adequate to determine whether or not albumin therapy affected the rate of ascitic fluid formation. It is true that in several patients fluid did not reaccumulate for several months after treatment, but these patients had exhibited similar remissions of ascites,

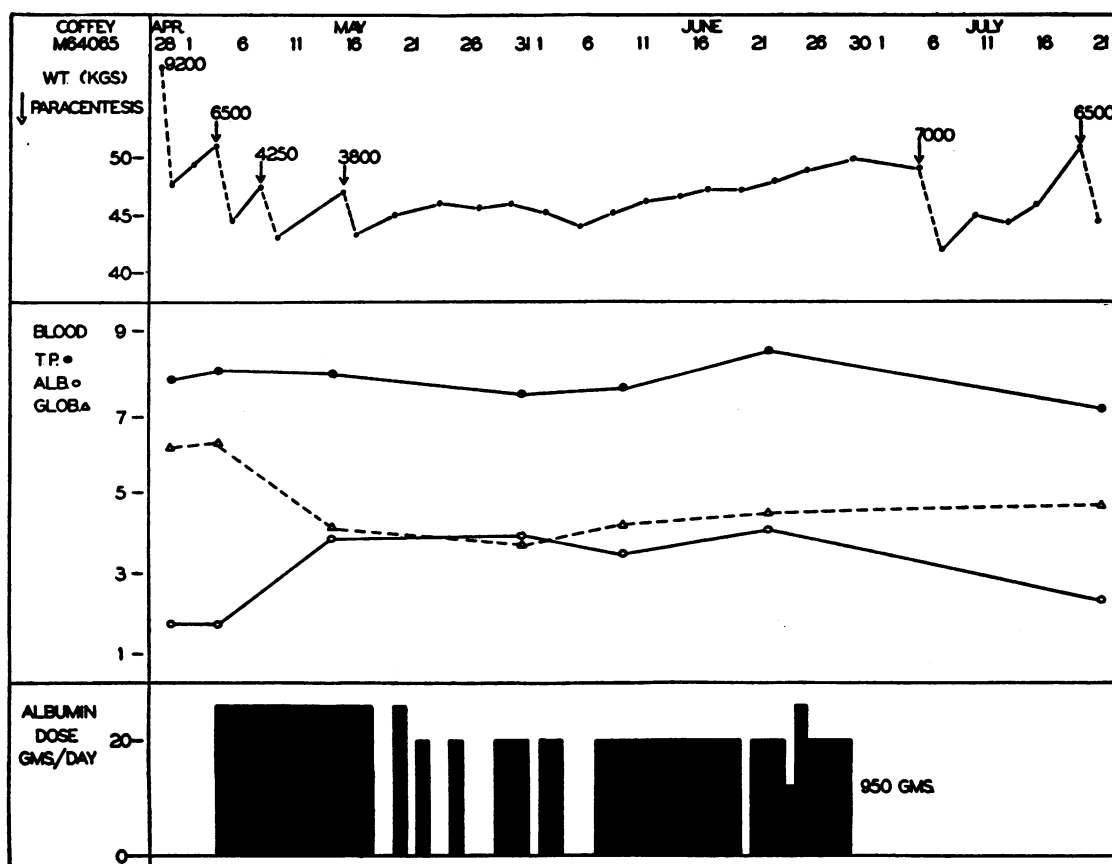


FIG. 8. CLINICAL COURSE OF PATIENT M. C. WITH CIRRHOSIS OF THE LIVER WHO RECEIVED 950 GRAMS OF ALBUMIN IN 8 WEEKS

Note sharp rise in serum albumin level at first and rapid fall after treatment was stopped.

previously. Our findings suggest that although ascites in these cases is usually associated with a diminished concentration of serum albumin, reduction in the colloid osmotic pressure of the blood is not the only factor in its production.

(c) *Patients with excessive protein loss.* Our third group of cases with hypoalbuminemia includes patients with proteinuria of sufficient degree to have depleted the plasma proteins. A few such cases have been treated, most of whom were in the nephrotic stage of chronic glomerulonephritis, while 2 children were classified as cases of true nephrosis, and another patient as a case of amyloid nephrosis. They were all similar in that they had low levels of serum albumin, they were losing large amounts of protein in the urine, and they were edematous.

Nephrotic edema has come to be regarded as the result of lowered colloid osmotic pressure of

the blood, due to loss of plasma proteins, particularly albumin, through the kidney. The logical assumption from this concept has been that if the total colloid osmotic pressure of the blood could be raised sufficiently, diuresis with loss of edema should occur.

Blood plasma and gum acacia have been widely used in the treatment of such cases with some success. A high protein diet has proved of value in aiding the synthesis of serum proteins and protecting the stores of the individual. It was felt that the injection of normal serum albumin might help to raise the level of this substance in the blood. It should be desirable to bring the serum albumin level to normal limits fairly rapidly, but due to its high osmotic activity, the amount of albumin which can be injected in a short period is definitely limited, since if given in excessive doses, the circulation

may become overloaded. For this reason, but mainly because the albumin testing program usually would not permit the administration of more than 1 bottle per day to a patient, we have restricted ourselves to doses of 25 grams per day in most instances. The albumin was given as a 25 per cent solution in all cases. This solution contained 0.85 per cent sodium chloride in cases 1 and 2, and 1.7 per cent sodium chloride in cases 3, 4, and 5.

Cases

(1) J. C., a girl aged 2, with chronic glomerulonephritis, was very edematous, her abdomen distended with fluid, and her eyes closed by swelling. She had previously been treated with blood, plasma, and urea. Three hundred grams of albumin were given in 10 to 25 gram doses, daily for 2 weeks. Her edema was reduced in amount and her weight decreased from 18.6 to 16.0 kilograms. Although her output was increased, the amount could not be measured. One determination of the urinary protein concentration, several weeks before treatment, was 5 Esbach units, and during albumin therapy it rose to 10 units, but the total excretion is not known. Her serum albumin and globulin levels were 1.5 and 1.6 grams per cent, respectively, before treatment, and these changed very slightly

to 1.8 and 1.2 grams per cent. When treatment was stopped because of lack of material, her weight promptly rose.

From this it was realized that very large doses of albumin would be necessary in these patients, as this child, weighing less than 16 kilograms, received 300 grams of albumin with only a slight effect on the serum albumin level.

(2) B. B., a patient with chronic glomerulonephritis in the nephrotic stage, was a 27-year-old male, who had had 2 previous admissions for the same disorder. On the first, he had been treated with plasma and acacia, and on the second, with casein hydrolysate. Each time there was a complete diuresis within a few weeks and he became asymptomatic for a period of months. The direct relation of treatment to diuresis could not be established. On this admission, his edema was much more persistent and he was in the hospital for more than 8 months before diuresis occurred. He was first given plasma and casein hydrolysate (1020 grams), but his weight continued to increase. In August 1942, his serum albumin level had fallen to 0.7 gram per cent, and on 2 consecutive days, his proteinuria amounted to 13 grams per day. During the next 22 days, he received 150 grams of plasma protein and 175 grams of albumin as shown in Figure 9. His serum albumin during this period rose to 1.0 per cent, with a somewhat greater

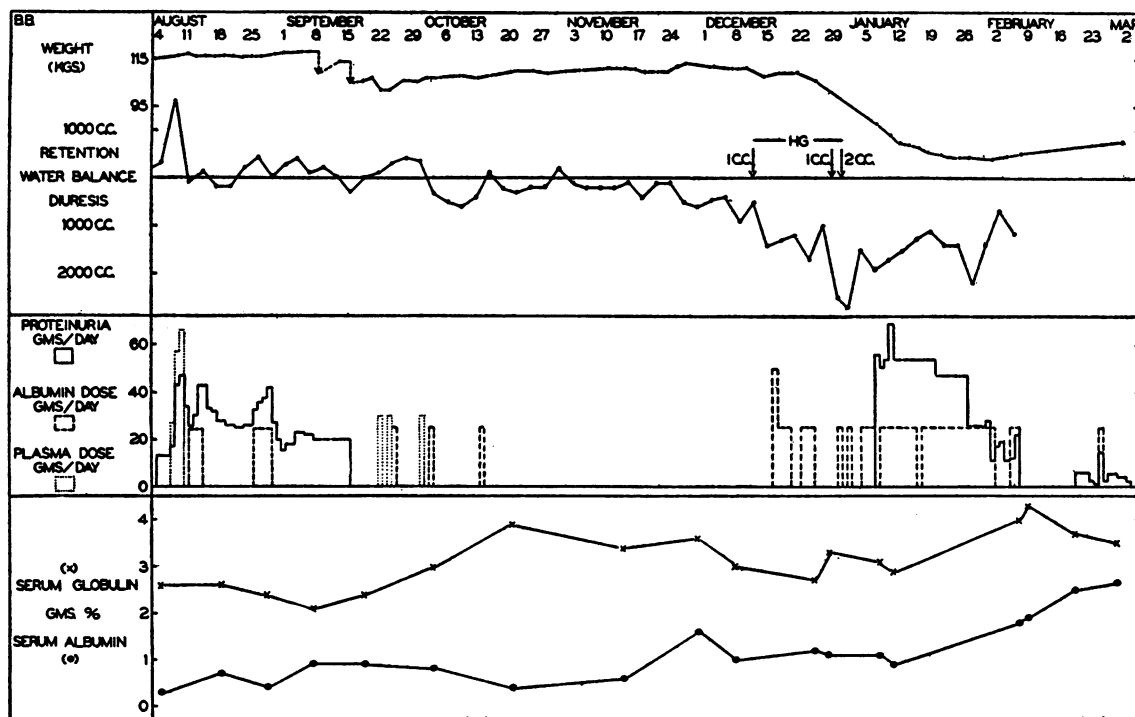


FIG. 9. CLINICAL COURSE OF PATIENT B. B. IN NEPHROTIC STAGE OF CHRONIC GLOMERULONEPHRITIS
Note increased proteinuria coinciding with injections of albumin or plasma.

increase in globulin, but the former soon fell to its previous low level. During the next 2½ months, his weight increased steadily (aside from the effects of 2 paracenteses), and he continued to retain water. From daily determinations of his protein excretion, it was found that immediately following each injection of protein, there was a marked increase in protein loss, so that within a few days, this appeared to account for the major portion of what had been given, although his nitrogen balance (Table V; I) had become somewhat more positive.

In late November, the patient's weight reached a maximum and started to decline. This was immediately preceded by a change to a consistent and increasingly negative water balance. When diuresis was well established, 300 grams of albumin were administered over a period of 12 days, but the serum albumin level did not change significantly. The urinary protein loss was again measured and found to be over 50 grams per day, a level which it maintained for the next 2 weeks, during which almost daily injections of 25 grams were given. When his weight had fallen and he became edema-free, the amount of protein excreted fell rapidly, over a period of several days. With this decrease, it became evident that the injected albumin was now being retained, and this was soon reflected by a sharp rise in serum albumin. The patient's diuresis was then complete. Two weeks later, his urine was found to contain only 3 to 5 grams of protein per day, but following one albumin injection of 25 grams, he appears to have excreted over half of it within 24 hours.

Our conclusion from this case was that the large doses of albumin injected were followed by a corresponding increase in protein excretion, and only when diuresis was nearly complete, and the degree of proteinuria had diminished, could the injected albumin be accounted for in the circulation.

(3) D. F., an 8-year-old boy, was admitted because of a puffy face and swollen scrotum. The urine contained large amounts of albumin and a very rare red cell. The edema was of a week's duration and had previously been present on only 2 occasions, lasting one day each. He was placed on a high protein, low salt diet, with restricted fluids, and diuresis commenced immediately, with a fall in weight for 3 days (Figure 10). It then stopped, however, and the weight was regained in a similar period. His serum albumin and globulin were 0.5 and 3.5 grams per cent, respectively, at this time. Albumin was then given intravenously for 30 days in doses of 20 to 25 grams per day, and his daily protein excretion increased markedly. Diuresis set in on the day albumin was started and his weight began to decrease. His serum albumin level for the first 10 days showed little change, despite the 225 grams of albumin administered. As the diuresis progressed, however, the proteinuria diminished sharply until it was evident that the injected albumin was being retained. Coincident with this decrease in protein excretion, his serum albumin level rose rapidly to normal. When seen

6 months later, he was found to have been free of symptoms, his urine contained no protein, and his serum protein value was 7.2 grams per cent, with an albumin level of 4.5 per cent.

(4) D. Fa.,¹² a girl of 21 months, with nephrosis, who had been in the hospital for several months without improvement, received 500 grams of albumin in a period of 20 days, an average daily dose of 25 grams. With treatment, there was a marked diuresis, disappearance of edema, and fall in weight from 12.6 to 8.4 kilograms, while her general condition improved markedly. Serum albumin and globulin values before treatment were 1.3 and 5.1 grams per cent, respectively, and afterwards 4.8 and 1.1 grams per cent. She was still improved clinically when seen 2 months later, but proteinuria was still present.

(5) E. H., a 27-year-old male, with chronic glomerulonephritis in the nephrotic stage, failed to improve despite the administration of a total of 565 grams in 15 to 25 gram doses, almost daily for 30 days. His weight increased steadily with continued accumulation of edema. His serum albumin level remained almost constant at 1.7 grams per cent, and marked proteinuria was present throughout his course. It was felt that he was doing so poorly that albumin should be discontinued.

One patient, B. F., suffering from generalized amyloid disease was given 250 grams of albumin in 10 days with an increase in serum albumin of only 0.5 gram per cent and some diuresis. Two other children in the nephrotic stage of chronic glomerular nephritis were treated. One, E. W., a 5-year-old child, received almost daily doses of 12 grams for 5 weeks without visible evidence of improvement, her serum albumin increasing from 0.9 to 1.2 grams per cent. The other, M. Ca., received 50 grams in 4 doses without effect except for an increase in proteinuria from 3.9 to 10 Esbach units, without change in urine volume. Treatment was stopped because of the difficulty in finding suitable veins. One adult patient, W. M., received over 1000 grams in a period of 6 months. During this time, his serum albumin rose only from 2.3 to 2.7 grams per cent, whereas his globulin rose from 1.5 to 2.8 grams per cent, and remission of edema occurred.

(d) *Miscellaneous cases.* Albumin has been given to a group of miscellaneous cases with edema from hypoproteinemia of relatively short duration. Six patients with burns who had developed edema with low serum proteins from excessive saline administration were treated 11

¹² This patient was treated by Dr. Raymond G. Filsinger, Buffalo Children's Hospital, Buffalo, N. Y.

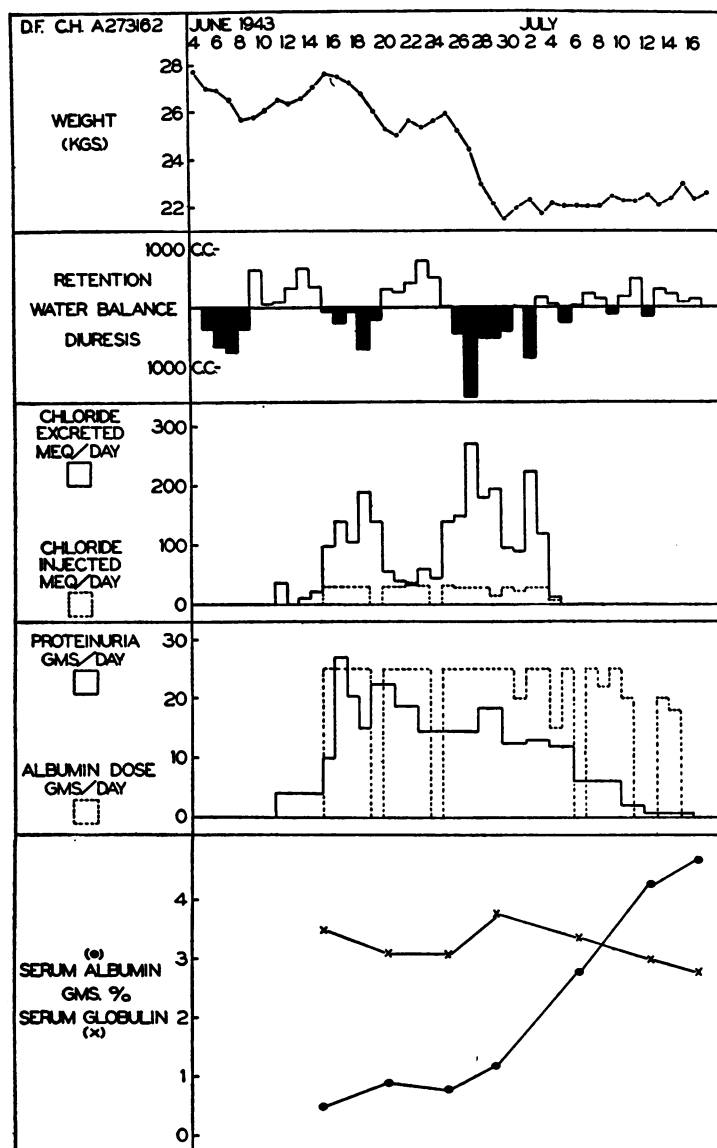


FIG. 10. CLINICAL COURSE OF 8-YEAR-OLD BOY (D. F.) WITH NEPHROSIS
Note failure of serum albumin level to rise appreciably until proteinuria diminished.

to 14 days after injury. The edema was reduced in each, and the serum albumin elevated in all but 1, whose diminished hematocrit reading showed that the plasma volume had probably increased. One patient with marked edema, as a result of the hypoproteinemia which followed a longer period of protein depletion after a very severe burn, received 200 grams of albumin and 1500 cc. of plasma in 6 days. This resulted in a marked diuresis, clinical improve-

ment, and a rise of serum albumin from 1.3 grams per cent with a total protein of 3.1, to 2.3 grams per cent with a total protein of 4.3. Four cases who developed edema during convalescence from intestinal surgery were treated. One of these, who received 195 grams in 7 days with elevation of serum albumin from 2.2 to 3.3 grams per cent, showed a considerable decrease in edema. Another experienced a reduction in edema following 25 grams and complete disap-

pearance after a second injection. One week later the edema had again set in and 25 grams was without effect. A third, receiving 50 grams, lost his edema, the serum protein increasing from 6.2 to 6.6 grams per cent, whereas a fourth case showed no effect on edema, serum protein, or hematocrit following 25 grams. In several patients with hypoproteinemia associated with suppuration, acute hepatitis, and carcinoma, the administration of albumin raised the serum protein level and aided in recovery when the condition was not hopeless. The doses used in these patients were 25 to 50 grams per day, except in a 7-month old baby who received 10 grams per day. In a patient, F. G., with long-standing hypoproteinemia associated with chronic constrictive pericarditis, 490 grams of albumin induced an increase in the retention of nitrogen (Table V; II) but failed to change her clinical condition or to alter the serum albumin level appreciably.

Discussion

Due to the specifications for concentrated normal human serum albumin, which required the clinical trial of each preparation before delivery to the armed forces, we have had a unique opportunity to use large amounts of this material in a few patients with hypoproteinemia. The requirements have, however, limited us to doses of no more than 25 grams per patient per day in most cases and to the treatment of patients in whom rises in temperature could be interpreted. This precluded the treatment of acutely ill patients in whom albumin might have produced more dramatic results and restricted us largely to those chronically ill.

The outstanding finding was that very large amounts of albumin were needed to raise the serum albumin level in patients with chronic hypoproteinemia, whether it was on a basis of malnutrition, altered synthesis as in cirrhosis of the liver, or proteinuria, as in the nephrotic syndrome. Since the amounts necessary to increase the circulating plasma proteins are so large, the use of albumin is chiefly indicated to tide the patient over an emergency. In malnutrition, the diet may be supplemented by protein hydrolysates given by mouth or by vein. However, if the serum proteins fall to edema

levels, intensive plasma or concentrated albumin therapy may be of value for a period.

In patients with cirrhosis of the liver with ascites, albumin therapy was found to have little to offer beyond temporary improvement in nutrition. The effect of raising the level of serum albumin on ascites was difficult to assess, but suggested that hypoalbuminemia is not the chief factor in its production.

In patients with severe proteinuria, a condition analogous to plasmaphoresis is set up, whereby the patient loses large amounts of albumin daily, indicating synthesis of plasma albumin and inability to retain it in the circulation. Since a considerable portion of injected albumin is lost in the urine, it is difficult to evaluate the effect of treatment, unless quantitative measurements of protein excretion are made, in order to determine how much albumin is actually retained. This amount seems to vary from one case to another, as Luetscher has shown (26), and at different times in the same patient (Figures 9 and 10). Our best results were obtained in 2 children with nephrosis, in whom the satisfactory clinical response may have been coincidental, or due to either the nature of their disease or to the larger doses given. Further studies, particularly in children, are justified on the basis of our experience, but only when sufficient albumin can be spared from the urgent needs of the armed forces. However, it is evident that enormous doses may be given without clinical improvement, that diuresis does not regularly result from albumin administration, and that when the albumin level in the plasma is temporarily raised by an injection of concentrated albumin, there is a corresponding increase in protein excretion.

Albumin has certain possible advantages over plasma for the treatment of hypoproteinemia. It is usually the deficient plasma protein in these cases. A large dose of protein can be administered in a small volume of fluid, a great asset in children. For the treatment of edematous patients, it could be dissolved in glucose solution instead of in 0.85 or 1.7 per cent sodium chloride, making it possible to administer the needed protein without salt. It should be stressed that concentrated albumin has not been shown to be a diuretic in 25-gram doses in adults. The evi-

dence from a few cases suggests that if 50 to 75 grams are administered in a short time to hypoproteinemic patients, producing a sufficient rise in colloid osmotic pressure with a more prolonged increase in plasma volume, some diuresis and diminution of edema may occur.

In the treatment of hypoalbuminemic patients, several factors must be kept in mind. Large doses are obviously necessary to produce an effect, and it would seem logical to administer a maximum amount of albumin in a minimum period of time. If the albumin is given much faster than it can be removed from the blood stream and metabolized or stored, venous congestion and excessive hemodilution may occur, as indicated by the course of one patient who received 650 grams in 6 days. Our experience suggests that the margin of safety is wide in most patients who do not have cardiac failure, but in attempting to raise the serum protein level rapidly, one should be guided by observations of the degree of venous and pulmonary congestion, and by determinations of hematocrit or hemoglobin level, in order to avoid overloading the circulation.

In addition, there are several considerations which should influence further studies on hypoproteinemic patients. It is quite possible that the various plasma globulin fractions may prove more useful in the treatment of certain forms of protein deficiency than albumin, since the fractions of plasma differ in amino acid composition, as shown by Brand (27), and in ability to promote plasma protein production in animals according to Cannon (28). Furthermore, in chronic protein depletion, the regeneration of tissue proteins is probably more important than the synthesis of the more readily measured plasma proteins.

It seems clear from our experience that concentrated albumin should have a useful place in therapeutics not only for the emergency treatment of shock, but for the treatment of a number of conditions in medicine and surgery, characterized by deficiency of serum albumin. Although we have had little opportunity to treat the acute and readily reversible types of hypoproteinemia, what experience we have had indicates that it is in this group of patients that albumin has most to offer. The rather disap-

pointing results in the patients with chronic hypoproteinemia due to the nephrotic syndrome or to cirrhosis of the liver is scarcely surprising and is in keeping with the observations of others who have had the opportunity to use large amounts of plasma in these conditions. Further careful studies on the possible therapeutic usefulness of albumin should be carried out, when the armed forces have been supplied with sufficient albumin to exceed their needs for the treatment of shock.

Summary of Part III

1. Evidence has been presented that concentrated human serum albumin is utilized like native serum albumin by hypoproteinemic patients.

2. Very large amounts of albumin were needed to raise the serum albumin level in patients with chronic protein depletion, whether on a basis of malnutrition, altered synthesis, as in cirrhosis of the liver, or chronic protein loss, as in nephrosis.

3. Only a small portion of the albumin retained could be accounted for in the circulation, and thus the major portion was presumably stored.

4. Although albumin did not appear in the urine in significant amounts after injection into patients with normal kidneys, a large part of that injected into 2 patients with the nephrotic syndrome was excreted.

5. It was possible to raise the level of serum albumin and decrease the level of serum globulin in patients with cirrhosis of the liver by repeated albumin injections, but this merely served to improve the patient's state of nutrition temporarily.

6. No conclusions are possible concerning the effectiveness of albumin in the treatment of nephrosis, but the results have been sufficiently encouraging in children to justify further careful investigations.

7. Although we have had few opportunities to treat cases of acute hypoproteinemia, the results suggest that albumin may ultimately prove most useful in this group of cases.

8. The main limiting factor in the rapid elevation of the serum protein level by albumin administration is the capacity of the circulation to adjust to increases in blood volume. Observations of the degree of venous and pulmonary

congestion and hemoglobin or hematocrit measurements should be used to guide therapy.

FINAL SUMMARY

Concentrated human serum albumin was developed to meet the needs of mobile military groups for a compact, stable, quickly available blood substitute for emergency use.

Part I deals with its use in shock. The standard Army and Navy package contains 25 grams of albumin in 100 cc. of diluent, and is equivalent to 500 cc. of citrated plasma in osmotic effect. When injected, it draws fluid rapidly into the circulation and has been successfully used in civilian hospitals to restore blood volume in 91 cases of shock due to trauma, hemorrhage, operations, and burns.

In Part II, the safety of albumin is discussed. Properly processed albumin does not produce reactions. Pyrogenic reactions were encountered in trials of certain lots, which were therefore rejected until the albumin was reprocessed to a satisfactory state. No evidence of sensitization to either native or heated albumin (50° C. for varying periods) was found. Neither clinical nor pathologic evidence of damage from repeated albumin injections was observed, except in one case in which pulmonary edema was attributed to overdosage (650 grams of albumin containing 1 : 10,000 merthiolate in 6 days).

In Part III, some data on the effects of repeated albumin injections in a small group of hypoproteinemic patients are reported. Albumin did not appear in the urine after injection unless there was proteinuria, nor was its injection followed by an increase in urinary non-protein nitrogen in hypoproteinemic patients, indicating assimilation, since only a small portion of the injected albumin could be accounted for in the circulation. Very large amounts given in doses of 25 grams daily were needed to produce an appreciable rise of serum albumin concentration in patients with chronic hypoproteinemia. In cirrhosis of the liver, albumin raised the serum albumin level but did not produce lasting benefit. In nephrotic patients, the injected albumin was largely excreted in the urine as protein; diuresis did not regularly result from albumin treatment but occurred coincidentally in 2 cases. There was little opportunity to treat patients with acute

hypoproteinemia, but the results in a few cases suggest that albumin ultimately will be most useful in this group.

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