GELATIN AS A PLASMA SUBSTITUTE: WITH PARTICULAR REFERENCE TO EXPERIMENTAL HEMORRHAGE AND BURN SHOCK*

W. M. PARKINS, PH.D., C. E. KOOP, M.D., C. RIEGEL, PH.D., H. M. VARS, PH.D., AND J. S. LOCKWOOD, M.D.

PHILADELPHIA, PA.

FROM THE HARRISON DEPARTMENT OF SURGICAL RESEARCH, SCHOOLS OF MEDICINE, UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA, PA

RESTORATION AND MAINTENANCE of circulating plasma volume are generally recognized as basic essentials in the prevention and treatment of shock. The successful use of crystallized human albumin indicates that the colloid osmotic pressure of plasma protein maintaining fluid distribution and plasma volume is a major physiologic factor in plasma and whole blood therapy.

In view of the difficulties of obtaining and preserving sufficient quantities of human plasma, it is natural to hope that the supply of plasma can be supplemented by a substitute which will be stable, innocuous, therapeutically effective, readily available, and reproducible with respect to its physiochemical properties. Many macromolecular colloids such as gum acacia, hemoglobin, bovine albumin, pectin, and a number of special gelatin preparations, including isinglass, have been, and are being, investigated. Hogan[1] reported, in 1915, that gelatin-saline infusions administered to patients in shock would restore falling blood pressure. Wolfson and Teller[2] found gelatin effective in experimental hemorrhage in the rabbit. Bayliss,[3] in 1917, and Rous and Wilson,[4] in 1918, strongly advocated the use of gum acacia, in preference to gelatin, in treatment of hemorrhage and shock. Although gum acacia has been used extensively, more recent study of its toxicity, especially with reference to its antigenicity, and its tendency to produce liver dysfunction and hypoproteinemia[5, 6, 7] has placed it in disfavor, except in emergency conditions when no other substitute is available.

Renewed interest in the possibilities of gelatin as a blood substitute is evidenced by a number of recent investigations (Taylor and Waters,[8] Waters,[9] Gordon, Hoge and Lawson,[10] Ivy, Greengard, Stein, Grodins and Dutton,[11] Little and Wells,[12] Ely and Angulo,[13] and Grodins.[14]

The aim of our study has been to determine: (1) The response of the

* The Charles B. Knox Gelatin Co. and Kind and Knox Gelatine Co., Johnstown, N. Y., and Camden, N. J., supplied the material, through the courtesy of Dr. D. Tourtellotte, and provided the University of Pennsylvania with a grant to defray a part of the cost of this study.

These studies were initiated by Drs. Norman E. Freeman and A. E. Schecter, who were forced to discontinue the work because of entering military service.

Preliminary reports of this work were presented at two conferences on gelatin, which were convened by the Subcommittee on Blood Substitutes of the National Research Council, in Washington, D. C., November 10, 1942, and February 23, 1943; and at the meeting of the Physiological Society of Philadelphia, April 20, 1943.

Read before the American Surgical Association, May 13–14, 1943, Cincinnati, Ohio.

normal dog to moderate and massive single and repeated infusions of bone collagen gelatin, with particular reference to general and specific toxic effects, many of which have been reported for other colloidal substances tested and used as blood substitutes; (2) the disappearance from the blood and excretion of gelatin; and (3) the efficacy of gelatin as compared with saline and plasma in the replacement of colloid osmotic pressure and plasma volume of dogs subjected to standardized conditions of hemorrhage or burn of a degree which is fatal to the untreated animal.

MATERIAL AND METHODS

The gelatin used in these studies was supplied as calcium gelatinate (Lot No. B78-1) produced by hydrolysis of alkali-treated bovine long-bone collagen, under controlled and standardized conditions. Our laboratory preparation was a six per cent solution in 0.85 per cent saline, sterilized by autoclave at 15 pounds pressure for 20 minutes. Aerobic and anaerobic sterility tests were uniformly negative. Although freshly prepared solutions were used routinely in these experiments, the preparation is stable for months when stored at 4°C. The solution after autoclaving has a p_H of 6; a specific viscosity of 1.6 at 37°C.; specific gravity of 1.024; and an osmotic pressure of 46 to 48 mm. Hg.

An 0.85 per cent saline solution was made from Sharp and Dohme sodium chloride tablets especially prepared for infusion purposes. The water was single-distilled and was neither tested nor treated for pyrogenicity. Fresh solutions were filtered and autoclaved at 15 pounds pressure for 20 minutes. Heparinized or citrated, pooled dog plasma was prepared from blood drawn under aseptic conditions from healthy animals.

All dogs used in these studies were carefully selected with regard to uniformity of nutritional state, age, size and general physical condition. They were acclimated to the laboratory and maintained under observation in individual cages for a period of at least one week prior to their use, and were fed a standard diet of Purina Checkers, with an occasional supplement of table or meat scraps.

Infusions were made by needle puncture through the jugular vein, and the apparatus was chemically clean and sterile. The solutions were kept at 32° to 37°C., and were administered at a rate of 0.5 to 1.0 cc. per kilogram per minute.

Blood pressure was determined in the femoral artery, by needle puncture, and mercury manometer and blood samples were withdrawn through the same needle.

Hematocrit determinations were made on heparinized blood in Wintrobe tubes, centrifuged at 2500 R.P.M. for 30 minutes. Plasma specific gravity determinations were made by the falling-drop method of Barbour and Hamilton.[15] Bromsulphalein retention was determined by the method of Rosenthal and White,[16] as modified by Helm and Machella.[17] Prothrombin time was measured by the procedure of Quick.[18] When plasma was analyzed

for plasma proteins and gelatin protein concentration, the differential precipitation of gelatin from plasma proteins was carried out with a modification of the method described by Waters.[16] The urine gelatin concentrations were determined by use of both tungstic and tannic acid preparation, and all nitrogen analyses were made with the aid of semimicro Kjeldahl apparatus.

EXPERIMENTAL STUDIES

I—INTRAVENOUS GELATIN IN NORMAL DOGS

1. *General Reactions.*—Following infusion of 30 and 60 cc. per kilogram of body weight (representing a 60 to 120 per cent increase in plasma colloid), all animals showed marked hemodilution, and with the larger doses, at least a temporary increase in blood pressure. The pulse and respiratory rates were increased for several hours after infusion. Nausea and emesis frequently occurred within two to three hours; and defecation occasionally followed within the same interval. Similar reactions often followed infusion of comparable volumes of saline or plasma. The animals usually appeared somewhat depressed and inactive for from three to six hours after the infusion of gelatin. By the following morning they were eager for food. Animals which received repeated infusions of gelatin, during a prolonged period, gained weight and remained in excellent general physical condition.

2. *Toxicity Studies*—(a) *Observations on Pyrogenicity.*—In 20 experiments upon ten normal dogs the temperatures were recorded at intervals of 15 minutes, 1, 2, 3, 4, 5, 6, and 24 hours after infusion of 30 cc. of gelatin per kilogram. At 15 minutes after infusion, the temperature was normal or somewhat below, with a slight increase by the second hour, reaching a peak between two and four hours, showing an average increase of 0.7° C. at three hours. The range was from no change to a maximum of 1.1° C. with a return to normal by the sixth hour. In four cases there was a slight temporary decrease in temperature. In four dogs infused with 10 cc. of gelatin per kilogram, no temperature rise was noted.

In four normal dogs subjected to massive hemorrhage (about 50 per cent of blood volume) followed by immediate infusion with an equal amount of gelatin, the temperature of one rose 1.0° C. in three hours, while that of two dogs dropped 1.1° C., and that of the fourth remained unchanged. Five dogs, bled in the same way (by needle puncture without anesthesia), and infused with sterile isotonic saline solution, showed a drop of 0.3° C. in one case, no change in two, and rise of 0.9° C. in the remaining two.

In view of the fact that the temperature changes with saline were similar to those with gelatin, when comparable volumes were administered, and considering the absence of temperature rises in the dogs receiving 10 cc. of glatin per kilogram, it appears doubtful that the gelatin *per se* is pyrogenic in the dog. Pyrogen tests in rabbits are in progress.

(b) *Observations on Antigenicity.*—Purified gelatin is generally considered to be nonantigenic.[19, 8, 9, 10] Sixteen normal dogs and five subjected to hemorrhage, were repeatedly infused with gelatin in amounts of 10 and

30 to 60 cc. per kilogram at intervals of days, weeks and months, without showing any evidence of anaphylaxis or sensitization as judged by blood pressure, respiration and other general reactions. Urticarial skin reactions commonly observed in dogs infused with dog plasma have in no instance been noted following infusion of gelatin. Animals have not yet been tested specifically for sensitization to other bovine protein.

(c) *Hematologic Observations:* Clotting Time, Pseudo-agglutination and Sedimentation Rate.—The clotting time of blood from dogs at various intervals after gelatin infusion (30 cc. per kilogram) was moderately increased. The average increase within 15 minutes was 16 per cent, and after three hours 30 per cent, with a return to normal within 24 hours. In two dogs subjected to triple hemorrhage and gelatin replacement, when a total of 90 cc. per kilogram was infused, the clotting time was about twice normal, and a return to normal occurred within 48 to 72 hours. Changes of the same order would be expected following infusion of similar volumes of citrated or heparinized plasma.

Pseudo-agglutination of erythrocytes has been reported to follow infusions of gelatin as well as other colloidal solutions used as blood substitutes.[20, 11, 14, 19] A pronounced increase in sedimentation rate after gelatin infusion was obvious to all who worked with the blood samples. Pseudo-agglutination and often massive clumping of erythrocytes were also apparent in the hematocrit blood samples, or when gelatin was mixed with red cells *in vitro*. However, the clumps which formed on standing were readily broken up with agitation.

Sedimentation rate determinations in gelatin-infused dogs, corrected for hematocrit changes, showed in most cases a two- to four-fold increase, with a return to normal as the gelatin disappeared from the blood stream. The increase in sedimentation rate was associated with, and apparently largely due to, the pseudo-agglutination of erythrocytes which occurred *in vivo* as well as *in vitro*. Direct observations and photomicrographs of this phenomenon *in vivo* were made by Dr. R. G. Abell, of the Department of Anatomy, using the ear-window technic in the normal unanesthetized rabbit. While the pseudo-agglutination of the red cells was present it did not appear to retard the circulation through the capillaries of the ear of normal rabbits infused with 15 cc. of gelatin per kilogram. The influence of this effect of gelatin, an effect which follows the administration of certain other colloids, upon the oxygenation of the blood and the peripheral circulation of animals in shock is deserving of further study, using special gelatin preparations.

(d) *Liver and Kidney Function.*—Observations on bromsulphalein retention, glucose tolerance, and prothrombin time, were undertaken as tests of liver function. Urea clearance was used as an index of kidney function.

Determinations were made before, and at intervals after, repeated weekly infusions of gelatin in 14 dogs. Three hours after each infusion of gelatin (30 cc. per kilogram) a marked retention of bromsulphalein was observed, which had diminished at 24 hours, and was essentially normal at 48 hours.

As illustrated in Figure 1, the degree of retention became increased, and the return to normal was increasingly delayed with each additional weekly infusion. The two dogs which showed the highest retention of bromsulphalein were selected for the illustration.

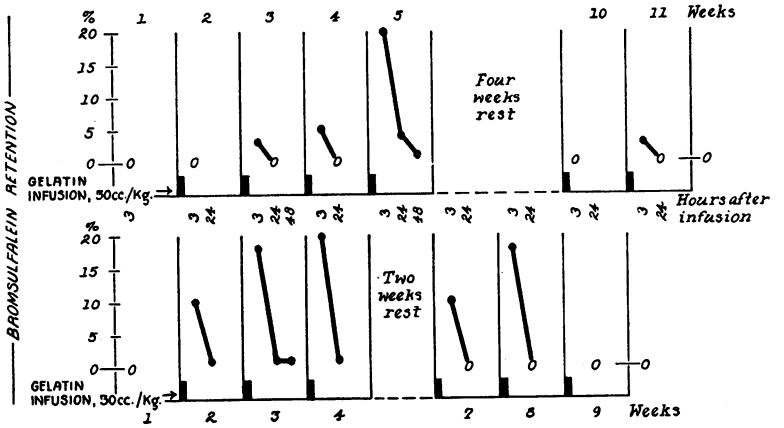

FIG. 1.—Bromsulfalein retention after weekly infusions of gelatin.

In animals in which the weekly infusions were suspended for a period of four or more weeks the retention of bromsulphalein after a subsequent infusion of gelatin was similar both in degree and duration to that following the first infusion of the series. However, the cumulative increase of bromsulphalein retention was present upon infusion following a rest of three weeks or less. It did not develop in dogs receiving weekly infusions of 10 cc. per kilogram.

Bromsulphalein retention was also determined after infusion of 30 cc. per kilogram of pooled heparinized dog plasma. Three of five dogs showed a bromsulphalein retention of as much as 16 per cent at three hours after a single plasma infusion.

The results of determinations of glucose tolerance after gelatin were variable, but no definitely abnormal curves were obtained.

The prothrombin time studies revealed normal values throughout except in one dog, which exhibited a prolonged time on two occasions as shown in Table I.

TABLE I

PROTHROMBIN TIME IN SECONDS

(Normal—under 21 seconds)

Weeks	208	243	273	296	300	
	9	17	13	9	13	Before first infusion
2	17			17	13	Between
3	14		13	14	14	3 and 4
4	(58)		12	11	11	hours
5		14				after
6	17	13		14		weekly infusions
7	(25)	14		16		of
8		13		16		gelatin
9					14	3% of body weight
10					16	
11		16				

The explanation of the bromsulphalein retention which follows massive gelatin and plasma infusions in normal unanesthetized dogs is not clear. In view of the transient nature of the retention after gelatin and plasma, it seems permissible to conclude that the liver dysfunction suggested by retention of bromsulphalein is probably not specific to gelatin itself and presents no serious obstacle to further clinical investigation of gelatin as a plasma substitute. It should be recognized that with an infusion of 30 cc. of gelatin per kilogram, the animal's plasma colloid would rise to about 160 per cent of normal unless temporary storage of gelatin or plasma protein occurred. It is during the period when a new colloid equilibrium is being reached that pseudo-agglutination of erythrocytes and retention of bromsulphalein are found. The repeated administration of smaller quantities of gelatin did not lead to cumulative retention of the dye.

(e) *Histologic Observations.*—With the collaboration of Dr. H. L. Ratcliffe of the Department of Pathology, there is in progress a thorough study of tissues from normal dogs and dogs subjected to hemorrhage, and from animals which succumbed to burn shock or were sacrificed at various intervals after infusions of gelatin, saline or plasma. A detailed report will be made upon completion of the observations. For the present, it can be stated that no evidence of chronic liver damage with storage of gelatin in the liver or other organs has been noted in normal dogs following single and repeated massive doses of gelatin. Tissue changes in liver, kidneys, and adrenals were only slightly more marked with gelatin than with plasma, and were definitely reversible.

II—DISAPPEARANCE FROM BLOOD, AND EXCRETION OF GELATIN

In a review of the literature on blood substitutes by Amberson,[19] it is concluded from publications on the use of gelatin preparations available 20 to 30 years ago that gelatin is relatively valueless because of the rapidity with which it leaves the blood stream. Waters[9] found calf skin gelatin to remain in the blood of infused animals much longer than does isinglass. Little and Wells[12] have recently shown that bone gelatin escapes through injured capillaries of the intestine more slowly than do the plasma proteins.

The basis of our interest in the rate of disappearance and the excretion of bone gelatin infused in normal dogs and in replacement of blood removed by a rapid massive hemorrhage was: (1) To correlate blood concentrations with other studies of efficacy and reactions to gelatin; (2) to determine whether the plasma proteins depleted by hemorrhage would be replaced in gelatin-infused animals at a rate sufficient to maintain total protein concentration, colloid osmotic pressure, plasma volume and blood pressure as the gelatin concentration decreased in the blood; and (3) to correlate the disappearance of gelatin from the blood with gelatin excretion, and with plasma protein concentrations, in the attempt to obtain information relative to the fate of gelatin and the question of its utilization as available protein.

Results.—Experiments on five normal dogs infused with 30 cc. of gelatin

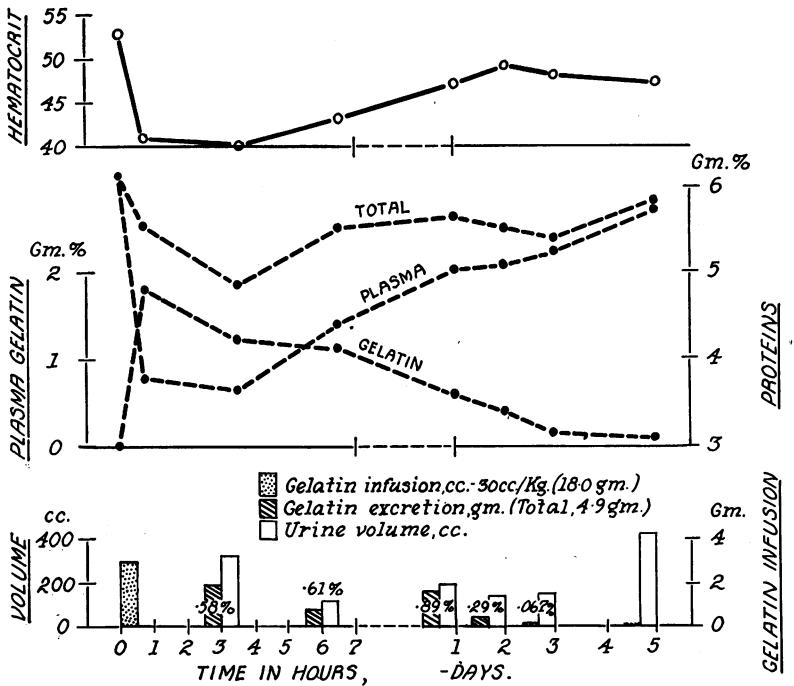

FIG. 2.—Gelatin disappearance from blood and gelatin excretion in normal dog.

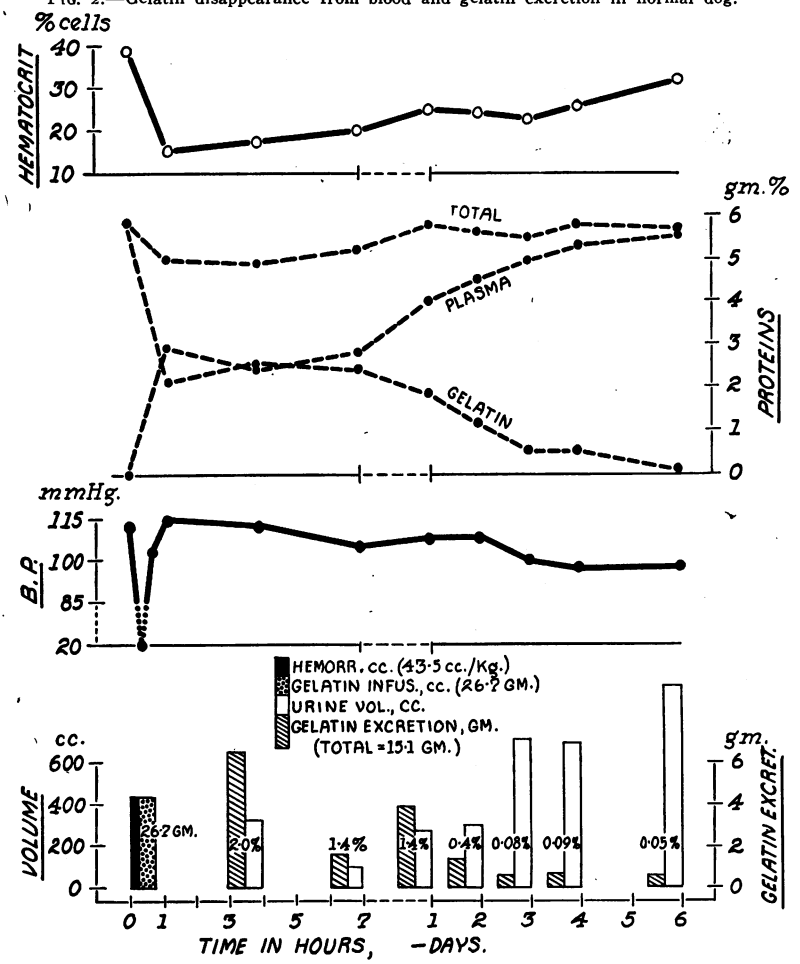

FIG. 3.—Gelatin disappearance from blood and gelatin excretion. Single massive hemorrhage.

per kilogram and on five unanesthetized dogs subjected to massive hemorrhage and infusion with a volume of gelatin equivalent to the volume of blood removed, are illustrated by representative data in Figures 2 and 3. It will be observed that a diuresis and relatively rapid excretion of gelatin occurred which decreased at six hours following infusion. Hemodilution and increased plasma volume, as indicated by hematocrit and plasma protein concentration, were well maintained in both the normal and the bled dogs, as was also the blood pressure following massive hemorrhage.

The plasma proteins were apparently replaced at about the same rate as that at which the gelatin disappeared from the blood stream. Following a brief period of osmotic adjustment to the high osmotic pressure of the infused gelatin, the total protein levels were well maintained in the experiments involving hemorrhage.

The decrease in total plasma protein concentration (including gelatin), which occurred soon after gelatin infusion, may be explained by increase in plasma colloid osmotic pressure and compensatory hemodilution, since the six per cent gelatin used has a colloid osmotic pressure about twice that of plasma. Determinations of plasma colloid osmotic pressure of blood drawn 15 minutes after gelatin replacement following hemorrhage, showed an increase of approximately 20 per cent.* Calculations indicate that the plasma volume is increased to an extent greater than the volume of fluid infused, which is in keeping with the increased osmotic pressure and consequent hemodilution from interstitial fluid.

Approximately one-half, or less, of the gelatin which leaves the blood stream is accounted for by the kidney excretion figures. The normal dog, in Figure 2, excreted 27 per cent of the gelatin infused; the dog subjected to hemorrhage (Figure 3), receiving a large amount, excreted 56 per cent. One other normal dog excreted 59 per cent.

Discussion.—The fate of gelatin which is not excreted remains obscure. Normal animals which received repeated weekly infusions of 30 cc. of gelatin solution per kilogram showed no indication of a deposition. Parenterally administered gelatin may be partially utilized in metabolism of the body tissues, or it may enter into maintenance or replacement of plasma proteins. That parenteral gelatin is metabolized to maintain nitrogen balance or equilibrium is indicated by the work of Brunschwig, Scott, Corbin and Moe.[21] Experiments now in progress will yield additional data relative to the question of the utilization of intravenously administered gelatin.

III—STUDIES ON HEMORRHAGE

COMPARISON OF GELATIN WITH SALINE AND PLASMA

Hemorrhage, by widely different procedures, and under various conditions, is commonly employed as a method by which the efficacy of potential blood substitutes may be evaluated. Unfortunately, there is no standard

* We wish to thank Doctors Tourtellotte and Williams, of the Kind-Knox Research Laboratories, for the colloid osmotic pressure determinations.

generally accepted procedure for this purpose. This fact makes difficult the comparison of results from different laboratories where various substitutes are under study.

Three bleeding procedures similar to those used experimentally by others, each of which is frequently parallelled in military or civilian injuries were employed in the following experiments. The efficacy of gelatin was compared under uniform conditions with saline or with saline and plasma. Conditions were such that few, if any, of the animals so treated would have survived without administration of one of the three infusion fluids.

I—SINGLE MASSIVE HEMORRHAGE AND IMMEDIATE INFUSION

Ten dogs were bled by arterial needle puncture, over a 10- to 15-minute interval, to the point of air hunger or respiratory failure, with the blood pressure falling to 15 to 20 mm. of Hg. Infusion was begun within two minutes. The volume infused was, in each case, equal to the volume of blood withdrawn. Four dogs, two in each infusion group, were given artificial respiration until the beginning of the infusion. Five animals were infused with gelatin, five were infused with saline.

Results.—The data are shown as average values in Table II. All these unanesthetized dogs survived the removal and replacement of approximately one-half of their total calculated blood volume. Obviously, survival as a

TABLE II

SUMMARY OF AVERAGE VALUES—SINGLE RAPID MASSIVE HEMORRHAGE
Immediate Infusion — Saline and Gelatin

Determination	Initial	Infusion	After Infusion					
			15 minutes		3 hours		24 hours	
				% Initial		% Initial		% Initial
Blood Pressure....	135	Gelatin	125	92.5	101	74.8	113	83.6
mm. Hg.....	134	Saline	90	67.0	105	78.4	118	88.0
Hematocrit.....	46	Gelatin	15	32.6	16	34.8	23	50.0
% cells.....	44	Saline	27	61.4	27	61.4	25	56.0
Total Protein	5.83	Gelatin	5.10	87.5	4.62	79.3	5.07	87.0
gm. %.....	5.22	Saline	3.21	61.5	3.74	71.5	4.28	82.0

criterion is of little value in determining the relative merits of infusion fluids under the seemingly severe conditions of hemorrhage in these experiments. A higher total protein concentration, a considerably greater decrease in hematocrit, and the return of blood pressure to normal within 15 minutes after gelatin infusion indicated a definite superiority of gelatin over saline in experimental hemorrhage of this type. Results from one such experiment in which gelatin disappearance and excretion were studied are illustrated by Figure 3.

DISCUSSION.—There is no general agreement in the literature regarding the efficacy of saline infusions in experimental hemorrhage.[22, 23, 24, 25, 26, 10, 11] It should be pointed out that animals show a remarkable spontaneous physio-

logic compensation to a massive rapid hemorrhage, if the brief critical period after hemorrhage is overcome by administration of any isotonic fluid. Even though the concentrations of plasma protein may be reduced, and the effect of saline in restoration of blood volume and pressure in some conditions may be transient, the administration of saline as a temporary expedient should not be ignored.

Conclusions as to therapeutic efficacy of a number of proposed blood substitutes have been based upon survival alone, or upon blood pressure elevation following infusion of a blood substitute in animals subjected to single rapid hemorrhage.[11, 8, 27] In those instances where the effect of saline alone was not determined under conditions of severe and otherwise fatal hemorrhage, the question may be seriously raised as to how much of the apparent influence of the blood substitute was a result of the fluid in which it was dissolved.

Under the conditions of single massive rapid hemorrhage and immediate infusion used in experiments illustrated in Table I, all of the dogs showed a definite hemodilution, increased blood pressure, and survived when saline or gelatin was infused. The early response to gelatin suggested its superiority over saline alone. However, the physiologic mechanisms of compensation were sufficient to make both groups more nearly comparable at three and 24 hours.

2—TRIPLE HEMORRHAGE AND INFUSION

A controlled triple hemorrhage procedure provided a more critical method of evaluating gelatin.

Dogs under nembutal anesthesia (25 mg. per Kg., intravenously) were bled 30 cc. per kilogram (approximately one-third their blood volume), and infused with an equal volume of gelatin, or saline. One hour after the first hemorrhage the animals were bled another 30 cc. per kilogram, or until the blood pressure dropped to 30 mm. of Hg. They were then infused. One hour later they were similarly bled and infused for the third time.

Results.—When saline was infused (Figure 4) the blood pressure returned to nearly normal levels after the first infusion, but was not well maintained and usually dropped to 30 mm. Hg. upon completion of the second hemorrhage. After the second infusion the blood pressure rarely returned to normal, and rapidly dropped to about 30 mm. of Hg. during the third hemorrhage. Only eight to 21 cc. per kilogram, with an average of 15 cc. per kilogram, could be withdrawn safely at the third bleeding, since a blood pressure below 30 mm. Hg. may be rapidly fatal. During the third infusion, the blood pressure rose to about 80 mm. of Hg. but was not sustained, even though additional saline may have been infused. However, two of the five dogs infused with saline recovered, even though the blood pressure remained at about 60 mm. Hg. for many hours. The plasma protein concentration was reduced to three to four Gm. per cent. The hemodilution

and increased plasma volume, as indicated by hematocrit and hemoglobin were well maintained.

The gelatin-infused dogs (Figure 5) were decidedly more resistant to this repeated bleeding. The blood pressure dropped but little more during the second hemorrhage than during the first; and in every case, the full

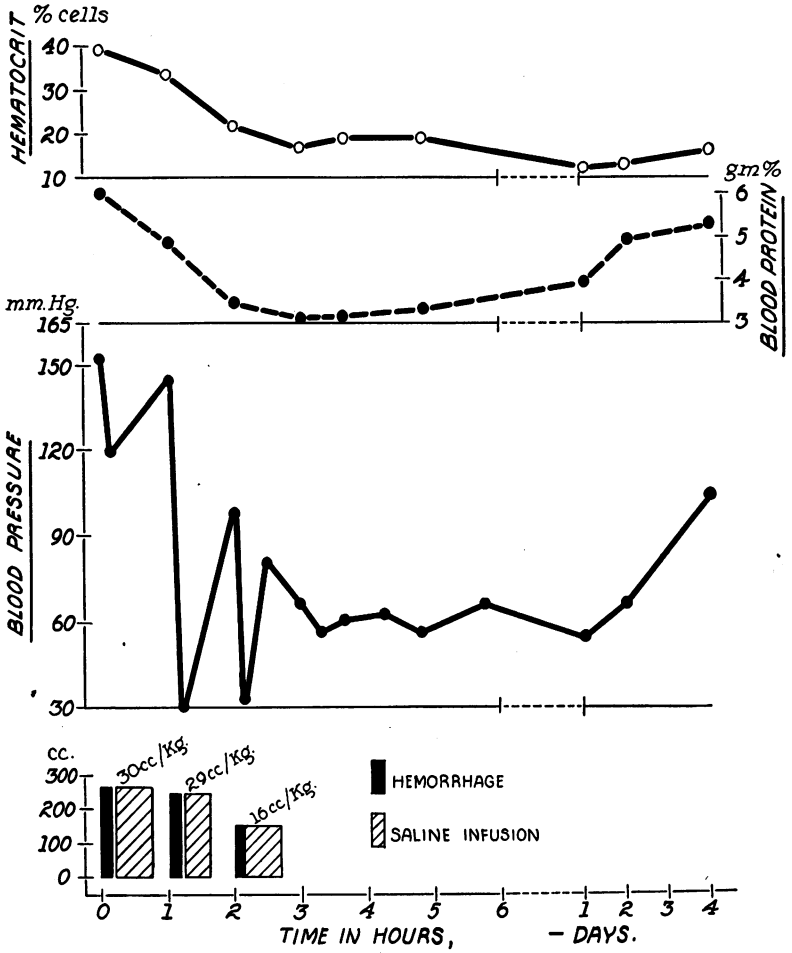

FIG. 4.—Saline in triple hemorrhage.

30 cc. per kilogram could be removed during the third hemorrhage without a decrease in blood pressure to critical levels. Three such dogs maintained a nearly normal blood pressure after the third infusion of gelatin, and recovered. With gelatin infusion the total protein levels were well maintained, and hemodilution was marked.

Two additional dogs were bled to a total of 100 cc. per kilogram, in three hemorrhages, followed by immediate infusions with gelatin. These two animals succumbed within 16 hours, even though the blood pressure fell to only 46 and 68 mm. of Hg., respectively, upon the third bleeding, and rose again to 74 and 80 after the third replacement with gelatin. Hematocrits

were 6.6 to 8.0 per cent, respectively, and the animals were markedly dyspneic. No erythrocytes were replaced in any of the triple hemorrhage experiments. It appears probable that death of these two dogs was due largely to anoxemia, resulting from a too drastic reduction in numbers of erythrocytes, rather than to depletion of plasma volume. The contrast between the gelatin and the saline-infused animals was most striking in the response to a third hemorrhage. Only eight to 21 cc. per kilogram, with an average of 15 cc., were bled from the saline-infused animals to reduce blood pressure to 30 mm.

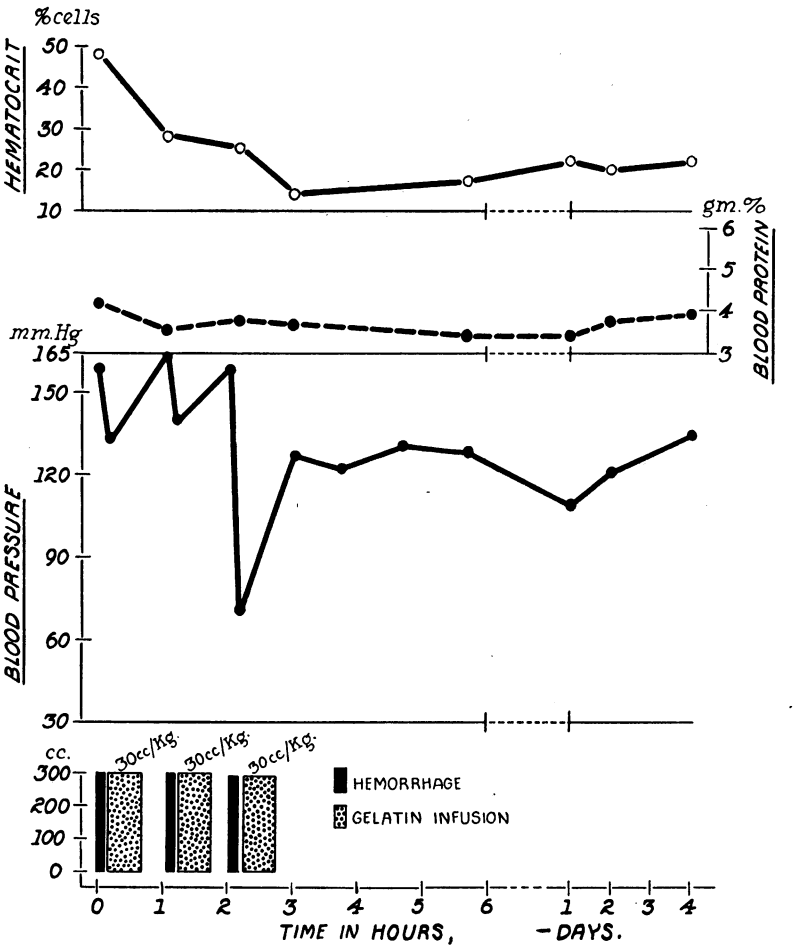

FIG. 5.—Gelatin in triple hemorrhage.

Hg. Thirty cubic centimeters per kilogram could be withdrawn during the third hemorrhage from each of the animals infused with gelatin, without reducing blood pressure to such a critical level.

The dog illustrated in Figure 6 showed definite and maintained improvement following gelatin infusions, although prior saline infusions had failed to restore stability of the blood pressure. Total protein concentration, hemodilution and blood pressure were increased and well maintained after

each gelatin infusion. Despite a blood pressure of 50 mm. of Hg., and below, for several hours during the period of saline infusion, the administration of gelatin was followed by complete recovery.

3—PROLONGED HEMORRHAGE AND SUSTAINED HYPOTENSION

The spontaneous compensation of the dog to hemorrhage, and the effectiveness of fluid replacement are markedly influenced by the rate of bleeding, and the degree and duration of the hypotension.[28, 29, 30, 31, 32] When the bleeding was prolonged or intermittent, and the blood pressure main-

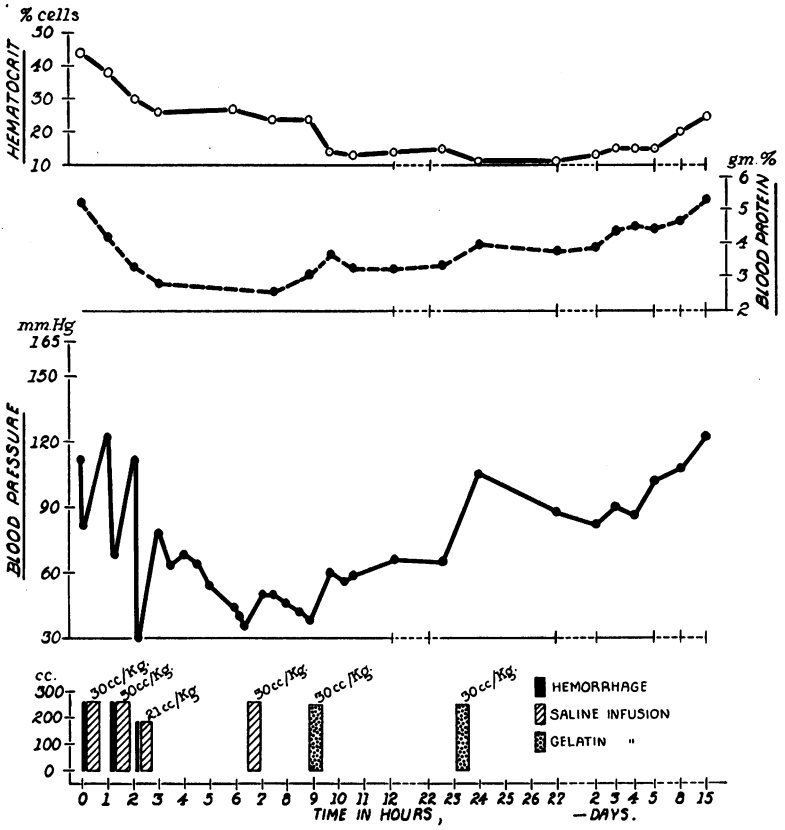

FIG. 6.—Saline-gelatin in triple hemorrhage.

tained at critically low levels by subsequent repeated withdrawals of blood, the response to a replacement of plasma volume is altered and the adequacy of the infusion fluid correspondingly diminished. Although hemodilution and increase in plasma volume and blood pressure occur after infusion of fluids, determining factors apart from blood volume in the blood pressure maintenance, such as peripheral resistance, may not be adequately compensated for by the infusion, and the circulation may fail.[30, 33]

In the following experiments gelatin as a plasma substitute was studied by comparison with saline and also with plasma under conditions wherein

saline was of little value in the majority of cases, and plasma was uniformly effective.

Sixteen dogs, under nembutal anesthesia, were bled in three stages, over a period of 30 to 40 minutes. Mean arterial blood pressure was maintained between 30 and 40 mm. Hg. for 30 to 40 minutes by additional bleeding if the blood pressure rose above 40. If the blood pressure dropped two

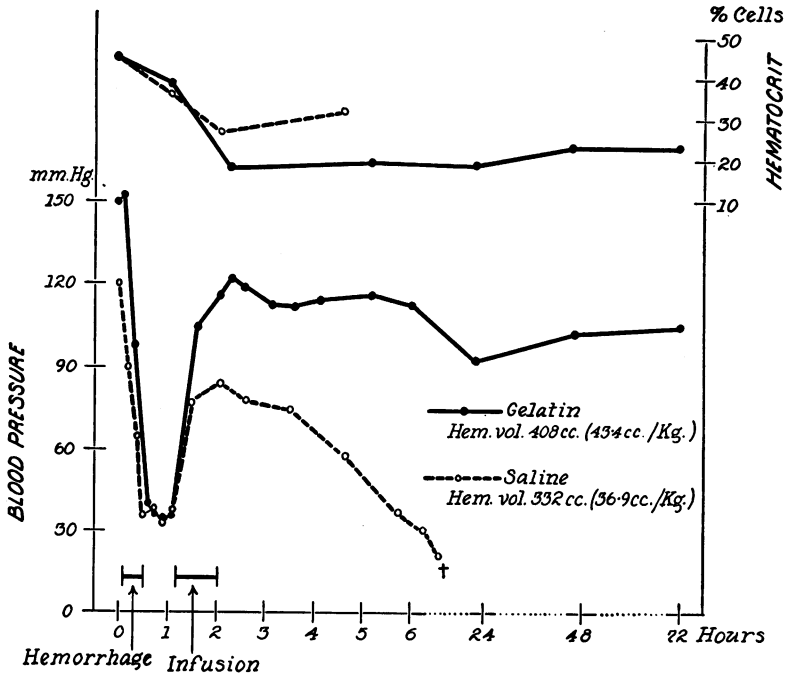

FIG. 7.—Gelatin and saline in hemorrhage.

or three millimeters below 30 mm. Hg. whole blood was injected in order to maintain the pressure at approximately 35. An infusion volume of the substitute equal to the volume of blood withdrawn (minus the amount replaced in keeping the animal alive during the hypotensive period) was administered at a uniform rate in each case.

Results.—Three untreated dogs succumbed within two and one-half hours. Three of five saline-infused dogs died within two to six hours; although the blood pressure after infusion was poorly maintained, two dogs recovered in spite of persistently low blood pressures of between 60 and 80 mm. Hg. for many hours after infusion. The blood pressure of all five gelatin-infused animals was increased to over 100 mm. Hg. upon injection of approximately 50 per cent of the infusion volume; it was further increased and well maintained following completion of the infusion (Figure 7). Complete recovery was rapid and uneventful in all five animals.

Three dogs infused with plasma reacted in much the same manner as did the gelatin-infused dogs, and all recovered. Figure 8 is typical of all

plasma and gelatin-treated animals. The hemodilution and increase in plasma volume, as indicated by hemoglobin and hematocrit, was more marked after gelatin infusion than after plasma. This may be due to the fact that the six per cent gelatin preparation used has a colloid osmotic pressure value

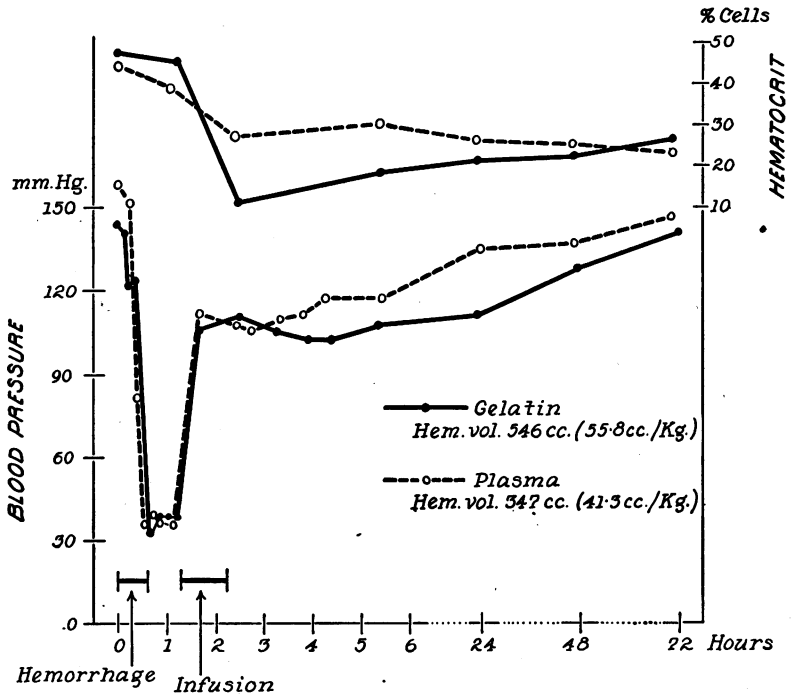

FIG. 8.—Gelatin and plasma in hemorrhage.

greater than that of plasma. Gelatin, under the conditions of this experiment, is undoubtedly superior to saline, and compares favorably with plasma as an effective blood substitute in maintenance of colloid ismotic pressure, blood volume and blood pressure. Gelatin thereby permits complete recovery of animals from a state of posthemorrhagic hypotension, which is fatal to untreated controls and to a majority of saline-infused animals.

DISCUSSION.—The question may be asked how gelatin compares with plasma and other plasma substitutes in experimental hemorrhage, under more drastic conditions where even plasma is not uniformly adequate for survival. Experiments now in progress, involving further prolongation of the period of hypotension, tend to indicate that under such extreme conditions the gelatin solution as now constituted falls short of being a complete replacement for plasma. Although the increase in blood volume may be even greater, and may be as well maintained after gelatin as after plasma, the blood pressure is more adequately sustained and survival more definitely assured with plasma than with gelatin. Hemodynamic factors, in addition to replacement of colloid osmotic pressure and plasma volume, enter into a consideration of complete recovery in these experiments.

In general, the results from a rather small number of dogs subjected to experiments on hemorrhage have led us to the tentative conclusion, that gelatin because of its colloid osmotic properties is definitely superior to saline, but may be deficient in some of the beneficial properties of whole blood or plasma. When tested under conditions where replacement of colloid osmotic pressure and plasma volume are sufficient, that is, before the exhibition of the so-called "irreversible" factors described by Wiggers[30, 33, 34] gelatin compares favorably with plasma.

IV—GELATIN IN BURN SHOCK

Although a given blood substitute may be found to be entirely satisfactory in the treatment of hemorrhage or posthemorrhagic shock it may or may not be equally efficacious in treatment of other shock states, such as those following thermal or mechanical injuries. Ely and Angelo[13] have concluded from their observations on rabbits subjected to sublethal burns, that a gelatin-glucose-salt solution is as effective as plasma in combatting the hemoconcentration. Grodins[14] recently reported that gelatin-saline is much more effective than saline alone, and about equal to plasma, in producing a sustained rise in blood pressure of dogs in shock resulting from limb trauma.

In studies of gelatin as a plasma substitute in burns, it seemed important to determine the hemodilution, blood pressure and other criteria of colloid osmotic pressure and fluid distribution in the first 12 to 24 hours, and also the later response of the treated animals in the so-called "acute toxemia" phase.

Eight of 30 dogs burned were used in trials to determine, by a controlled reproducible procedure (similar to that described by Trusler, Egbert and Williams[35]), the degree of injury which would be fatal to most if not all untreated animals; a burn not so severe as to be irreversible, but one in which plasma therapy might still be effective. The following procedure was used in the 22 dogs included in this report: The hair of the animal was closely clipped from the hindquarters and body, up to the axillae. The animal was anesthetized with morphine and amytal, then immersed up to the axillae in water at 72° C. for 60 seconds. Ether was used to supplement the anesthesia during the immersion. Observations were continued to death, or for a period of 72 to 96 hours of survival, at which time the recovering animals were sacrificed for pathologic study. All dogs were infused with 30 cc. per kilogram of body weight of the test solutions, at comparable intervals of time (two and nine hours) following the injury. The volume of the third infusion varied between 1.5 per cent and 3.0 per cent of body weight, depending upon the extent of the hemoconcentration at the time. Other conditions were kept uniform, including the taking of blood samples and blood pressure determinations. The animals were permitted to recover from the anesthesia. Sulfathiazole ointment was used to control skin infections.

Results.—The average survival time of five untreated animals was 11 hours, with a range of from four to 19 hours. Of the five saline-treated animals, two recovered, and were sacrificed at 72 hours, while three succumbed within nine to 20 hours, with an average survival of 13 hours. One gelatin-infused dog recovered; seven died in from nine to 67 hours, with an average survival of 28 hours. Three of the four plasma-treated dogs recovered; one of the plasma animals died at nine hours, just previous to the second infusion.

Although the hemodilution effect of saline was slight in comparison with gelatin or plasma, it appeared that saline alone was beneficial to these animals. Two dogs survived the first 12- to 24-hour period of critical plasma leakage, reached the stage when compensatory blood volume control was reestablished, and hemoconcentration spontaneously disappeared while the blood pressure was maintained.

The rapid progressive hemoconcentration, due essentially to leakage of plasma into the injured area through capillaries permeable to protein, was well compensated by gelatin infusions. Figure 9 is representative of typical cases in each of the four groups of animals. The colloid osmotic pressure of the plasma protein appeared to be adequately replaced by gelatin, since the hematocrit and hemoglobin levels could be brought to normal, and were about as well maintained as with the infusion of equivalent volumes of plasma, as shown by Figure 9 and Table III. The rate of hemoconcentration following the first and second infusions of gelatin, as compared with that following plasma infusion, suggests that the gelatin molecules may be as well retained by permeable capillaries as are the plasma proteins.

Discussion.—With gelatin infusions the survival of the animals could be extended beyond the period when plasma loss into the burned area might be considered to be a cardinal factor. Although the hemoconcentration was corrected, the blood pressure was not permanently maintained and, with the

Table III

SUMMARY OF AVERAGE VALUES
HEMOCONCENTRATION (HEMATOCRIT) OF DOGS SEVERELY BURNED

Treatment	No. of Dogs	Hematocrit (% cells) Normal	Per Cent of Normal			
			Infusion I		Infusion II	
			Before	After	Before	After
Saline	5	44	136	126	153	127
Gelatin	8	42	137	105	145	95
Plasma	4	45	140	116	149	94

exception of one dog, the animals, nevertheless, succumbed. The mechanism involved in this circulatory collapse which is unassociated with hemoconcentration in the secondary phase of burn shock remains obscure. The possibility of functional impairment of some vital organ such as liver, adrenal or kidney,

already damaged through anoxia or through absorption of some hypothetic toxic substance from the burned area, has not been excluded.

Since gelatin compensates for the osmotic pressure of lost plasma protein and apparently corrects the plasma volume deficiency of burn shock, we are determining whether or not the efficacy of gelatin in burn treatment may be enhanced through modification of the gelatin solution or its technic of administration, and through the concomitant use of accessory humoral factors.

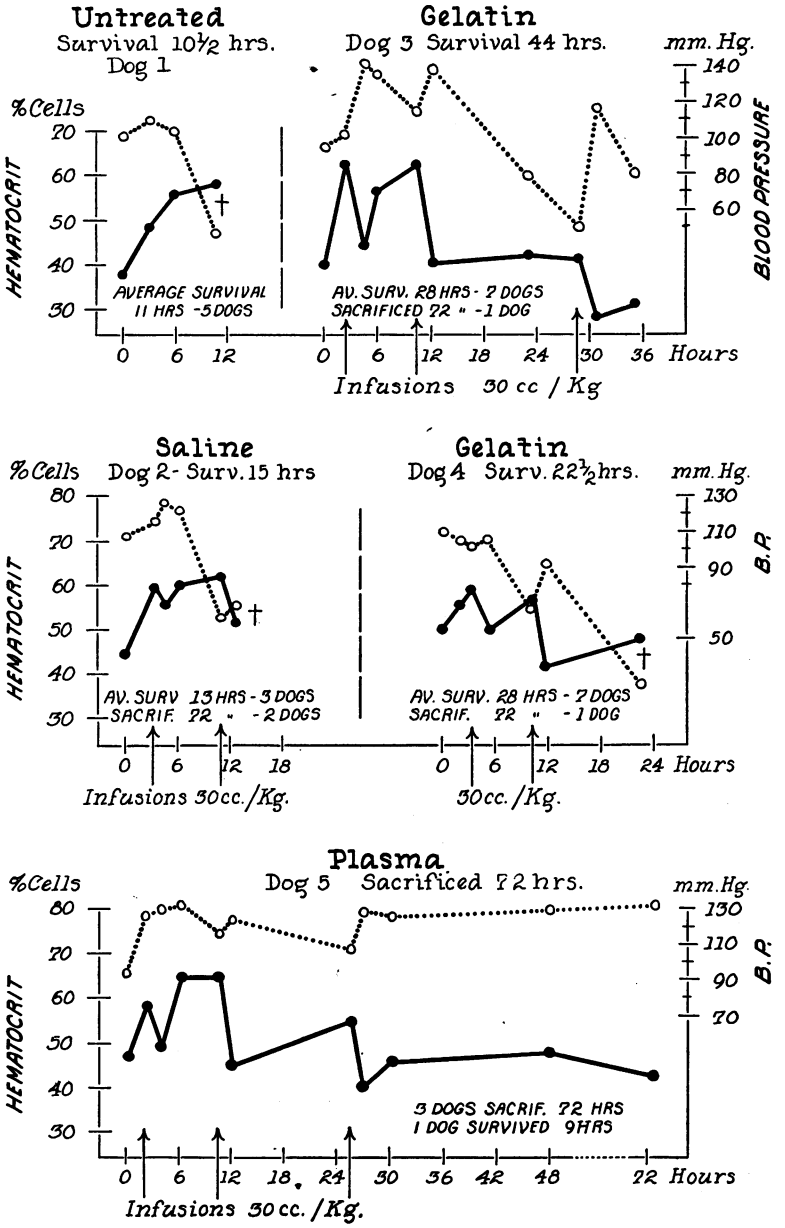

FIG. 9.—Saline, gelatin and plasma in burn shock.

SUMMARY AND CONCLUSIONS

1. Normal dogs tolerate repeated infusions of large volumes of gelatin-saline solution without any serious toxic reactions specific to gelatin. Unfavorable reactions that do occur are reversible and are frequently produced by infusions of comparable amounts of saline or plasma.

2. Pseudo-agglutination of erythrocytes and increase in sedimentation rate, which also occur following infusion of other macromolecular colloids, have been observed both *in vitro* and *in vivo* following infusions of gelatin.

3. Bromsulphalein retention occurred after repeated infusions of gelatin but is present to some extent, in dogs infused with comparable volumes of plasma. Other liver function tests were essentially negative.

4. Kidney function, as determined by urea clearance, was apparently unimpaired after single and repeated infusions of gelatin in normal dogs.

5. Tissue changes observed histologically were reversible and slightly more marked with gelatin than with plasma infused in equivalent volumes and under similar conditions. No chronic tissue storage was observed in animals which received repeated infusions of large volumes of gelatin.

6. A transient diuresis and excretion of gelatin followed its infusion in normal dogs and dogs subjected to preliminary hemorrhage. About half of the gelatin infused was accounted for by urinary excretion.

7. Following massive, rapid hemorrhage and immediate infusion of gelatin, the plasma proteins were apparently replaced at about the same rate as that at which the gelatin protein disappeared from the blood stream.

8. All dogs survived a rapid, massive hemorrhage to point of respiratory failure and blood pressure levels of approximately 20 mm. Hg. when immediately infused with either saline or gelatin. The higher total protein concentration following gelatin infusion, the considerably greater hemodilution, and the more rapid return of the blood pressure to normal, strongly bear out the superiority of gelatin over saline in experimental hemorrhage of this type.

9. The resistance of the dog to repeated massive hemorrhage, followed by gelatin infusion is markedly increased over that of the animal similarly bled and infused with saline. The saline-infused dog in which the blood pressure continued to decline and remained at critical levels for hours following repeated infusions completely recovered upon subsequent infusions of gelatin.

10. Following a slow, three-stage hemorrhage, with blood pressure maintained at 30 to 40 mm. Hg. for 30 to 40 minutes, all untreated dogs succumbed within a few hours. Although elevation of plasma volume and blood pressure were not marked or well maintained after infusion of saline, compensation occurred and two of five dogs slowly recovered. Three of the saline-treated animals died in two to six hours. Gelatin infusion resulted in a more marked hemodilution than did plasma infusion under uniform volume replacement. The blood pressure was restored and main-

tained at approximately the same normal levels with gelatin as with plasma, and all dogs made a rapid, complete recovery under these conditions of experimental hemorrhage, gelatin appears to be a suitable substitute for plasma.

11. In eight dogs subjected to a standardized, reproducible burn of a degree fatal to untreated animals and to a majority of saline-infused animals, gelatin solutions compensated for the loss of plasma from the blood stream, and corrected hemoconcentration to the same degree as did plasma infused in four animals. Although survival was extended in the burn shock gelatin group beyond the period when death occurred in controls, presumably from hemoconcentration, seven of eight dogs succumbed after progressive decline in the blood pressure, unassociated with hemoconcentration. Under identical conditions, with plasma infusion, only one of four dogs succumbed.

12. If a factor can be identified in plasma which accounts for its ability to maintain blood pressure in the severely burned animal during the secondary phase of so-called "acute toxemia," the addition of this factor to gelatin would probably result in a more adequate plasma substitute for burns.

REFERENCES

1 Hogan, J. J.: The Intravenous Use of Colloidal (Gelatin) Solutions in Shock. J. A. M. A., **64**, 721, 1915.

2 Wolfson, W. L., and Teller, F.: The Intravenous Use of Gelatin Solution in Hemorrhage. Am. J. Med. Sci., **178**, 562, 1929.

3 Bayliss, W. M.: Methods of Raising a Low Arterial Pressure. Proc. Roy. Soc. London, Ser. B, **89**, 380, 1917.

4 Rous, P., and Wilson, G. W.: Fluid Substitutes for Transfusion after Hemorrhage. J. Am. Med. Assoc., **70**, 219, 1918.

5 Maytum, C. K., and Magath, T. B.: Sensitivity to Acacia. J. A. M. A., **99**, 2251, 1932.

6 Andersch, M., and Gibson, R. B.: Studies on the Effects of Intravenous Injections of Colloids. I. Deposition of Acacia in the Liver and Other Organs and its Excretion in Urine and Bile. J. Pharm. and Exper. Therap., **52**, 390, 1934.

7 Yuile, C. L., and Knutti, R. E.: Blood Plasma Proteins as Influenced by Intravenous Injection of Gum Acacia; S. Production of Chronic Hypoproteinemia. J. Exper. Med., **70**, 605, 1939.

8 Taylor, N. B., and Water, E. T. Isinglass as a Transfusion Fluid in Hemorrhage. Canad. Med. Assoc. J., **44**, 547, 1941.

9 Waters, E. T.: A Comparison of Isinglass and Gelatin as Blood Substitute. Canad. Med. Assoc. J., **45**, 395, 1941.

10 Gordon, H., Hoge, L. J., and Lawson, H.: Gelatin as a Substitute for Blood After Experimental Hemorrhage. Am. J. Med. Sci., **204**, 4, 1942.

11 Ivy, A. C., Greengard, H., Stein, I. F., Jr., Grodins, F. S., and Dutton, D. F.: The Effect of Various Blood Substitutes in Resuscitation After an Otherwise Fatal Hemorrhage. Surg., Gynec. and Obst., **76**, 85, 1943.

12 Little, J. M., and Wells, H. S.: Capillary Permeability to Intravenously Administered Gelatine. Am. J. Physiol., **138**, 495, 1943.

13 Ely, J. O., and Angulo, A. W.: Experimental Burns: The Influence of a Gelatin-Glucose-Salt Solution on the Hemoconcentration of Burns. J. of the Franklin Instit., **235**, 197, 1943.

14 Grodins, F. S.: Gelatin as a Blood Substitute in Shock due to Limb Trauma. Federation Proceedings, **2**, 17, 1943.

15 Barbour, H. G., and Hamilton, W. F.: The Falling-Drop Method for Determining Specific Gravity. J. Biol. Chem., **69**, 625, 1926.

16 Rosenthal, S. M., and White, E. C.: Clinical Application of the Bromsulphalein Test for Hepatic Function. J. A. M. A., **84**, 1112, 1925.

17 Helm, J. D., and Machella, T. E.: Significance of Dosage and Time Factors on the Value of the Bromsulphalein Test for Liver Function. Am. J. Digest. Dis., **9**, 141, 1942.

18 Quick, A. J.: The Clinical Application of Hippuric Acid and Prothrombin Tests. Am. J. of Clin. Path., **10**, 222, 1940.

19 Amberson, W. R.: Blood Substitutes. Phys. Rev., **12**, 48, 1937.

20 Hanzlik, P. J., and Karsner, H. T.: Anaphylactoid Phenomena from Intravenous Administration of Various Colloids, Arsenicals and other Agents. J. Pharm. Exp. Therap., **14**, 379, 1920.

21 Brunschwig, A., Scott, V. B., Corbin, N., and Moe, R.: Observations on the Intravenous Injection of Gelatin for Nutritional Purposes. Proc. Soc. Exp. Biol. and Med., **52**, 46, 1943.

22 Blalock, A., and Beard, J. W.: The Effects on the Composition of the Blood of the Subcutaneous Injection of Normal Salt Solution into Normal Dogs and into Dogs Submitted to Intestinal Trauma, Graded Hemorrhages, and Histamine Injection. J. Clin. Invest., **11**, 311, 1932.

23 Beard, J. W., Wilson, H., Weinstein, B. M., and Blalock, A.: A Study of the Effects of Hemorrhage, Trauma, Histamine, and Spinal Anesthesia on the Composition of the Blood when no Fluids are Injected and when Fluids are Injected Intravenously. J. Clin. Invest., **11**, 291, 1932.

24 Hoitink, A. W. J. H.: Treatment of Acute and Fatal Hemorrhage by Injection of Artificial Blood Substitutes: A Comparative Study of Artificial Blood Substitutes. Surg., Gynec. and Obst., **61**, 613, 1935.

25 Buttle, G. A. H., Kekwick, A., and Schweitzer, A.: Blood Substitutes in Treatment of Acute Hemorrhage: An Experimental Evaluation. Lancet, **2**, 507, 1940.

26 Harkins, H. N., and McClure, R. D.: The Present Status of Intravenous Fluid Treatment of Traumatic and Surgical Shock. ANNALS OF SURGERY, **114**, 891, 1941.

27 Hartman, F. W., Schelling, V., Harkins, H. N., and Brush, B.: Pectin Solution as a Blood Substitute. ANNALS OF SURGERY, **114**, 212, 1941.

28 Blalock, A.: Shock; Further Studies with Particular Reference to the Effects of Hemorrhage. Arch. Surg., **29**, 837, 1934.

29 Freeman, N. E., Shaffer, S. A., Schecter, A. E., and Holling, H. E.: The Effect of Total Sympathectomy on the Occurrence of Shock from Hemorrhage. J. Clin. Invest., **17**, 359, 1938.

30 Wiggers, C. J.: The Present Status of the Shock Problem. Physiol. Rev., **22**, 74, 1942.

31 Wiggers, C. J., and Werle, J. M.: Exploration of a Method for Standardizing Hemorrhagic Shock. Proc. Soc. Exp. Biol. and Med., **49**, 604, 1942.

32 Ebert, R. V., Stead, E. A., Jr., Warren, J. V., and Watts, W. E.: Plasma Protein Replacement after Hemorrhage in Dogs with and without Shock. Am. J. Physiol., **136**, 299, 1942.

33 Wiggers, C. J., and Werle, J. M.: Cardiac and Peripheral Resistance Factors as Determinants of Circulatory Failure in Hemorrhagic Shock. Am. J. Physiol., **136**, 421, 1942.

34 Wiggers, C. J.: Recent Studies on the Irreversibility Characteristics of Shock. Exp. Med. and Surg., **1**, 2, 1943.

[35] Trusler, H. M., Egbert, H. L., and Williams, H. S.: Burn Shock: The Question of Water Intoxication as a Complicating Factor: Blood Chemical Studies and Report of an Extensive Burn Treated by Repeated Transfusions of Blood and Blood Plasma. J. A. M. A., 113, 2207, 1939.

DISCUSSION.—DR. JOHN S. LOCKWOOD (Philadelphia, closing): I want to thank Dr. Graham for making reference to Hogan's work on gelatin. As a matter of fact, we have referred to it in the manuscript, but because of the shortage of time I omitted reference to it, which I would not have done had I been aware of the fact that that work was done in Cincinnati. Gelatin chemistry has made many advances toward purification and standardization of material, which gives us many advantages over workers of twenty-five years ago.

I also omitted reference in my remarks to the very interesting study being conducted by the group in Chicago, under Dr. Brunschwig. We feel that these two studies are supplementing each other in a very useful way. We also believe that gelatin may serve as a means of providing parenteral feeding of protein.

One of the problems we have been up against all along in this work has been to "hew to the line" of studying gelatin as a blood substitute, because so many interesting problems concerning shock and burns have cropped up in connection with the work, that we have been tempted frequently to drop our primary study in pursuit of these possible "will-o'-the-wisps." For example, we think the gelatin work has brought out further evidence of the dissociation of the problem of blood-volume maintenance from the problem of peripheral blood flow in the shocked individual.

The restoration of normal blood volume by the administration of a colloid such as gelatin will not alone constitute adequate therapy in shock.