THE EFFECT OF SERUM TRANSFUSION ON THE PLASMA PROTEIN DEPLETION ASSOCIATED WITH NUTRITIONAL EDEMA IN DOGS

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The important physiologic rôle of the blood serum albumin in maintaining normal water distribution between blood vessels and interstitial spaces has prompted an interest in therapeutic measures which might be available for the relief of patients with serum albumin deficits. The most obvious means of attempting to replenish such deficits is by transfusion with normal serum. It would further seem that to be effective these transfusions would have to be relatively large in volume. A normal individual who weighs 70 kilograms has about 150 grams of albumin circulating in his blood vessels.[1] The same individual with a supply of albumin depleted to the level at which edema first occurs would have about 90 grams. To replace the deficit by transfusion would require approximately 1400 cc. of normal serum and about 3 liters of blood would be required to furnish the serum. Furthermore, since the symptoms of serum albumin deficit are more closely related to concentration than to the total amount in circulation, it follows that transfusion with the volume of serum just mentioned would accomplish its purpose only if it could be given without a change in blood volume. If it were attended by any considerable increase in blood volume, much larger amounts would be required. Because of the possible untoward effects of the transfusions, because a proper understanding of the effects of the therapy necessitates frequent blood sampling, and because of the expense involved in obtaining large amounts of serum from donors, it has seemed wise to study the various reactions in experimental animals before attempting to treat human patients. We wish to report here the results obtained in dogs.

The general plan of investigation involved giving relatively large serum transfusions to dogs, which at the time exhibited serum albumin deficits, and noting the changes produced in the plasma proteins both as an immediate result of the procedure and during subsequent days. The method of producing albumin depletion has been described in a previous paper (1). Eight transfusions in five dogs have been studied. In

[1] Plasma volume estimated at 50 cc. per kilogram; serum albumin at 4.3 grams per 100 cc.

animals which had been subjected to plasmapheresis experimental observations on the effect of transfusion were not made until sufficient time had elapsed after the last bleeding (14 to 30 days) to permit reestablishment of relatively stationary protein levels. Five of the transfusions were given during periods when edema was present, three when there was no edema. Dogs 448 and 772, without edema, received one transfusion each. Dog 589 received two transfusions which were separated by an interval of 15 days. Edema was absent when the first was given but present with the second. Dog 449, with edema, received three transfusions on consecutive days. Unfortunately, during the night following the first transfusion, a huge hematoma developed in the left groin at the site of the puncture made for sampling. Because of the hemorrhage the second transfusion was begun with a plasma protein concentration lower than that which had been present before the first. Dog 451, with edema, received a single transfusion which was given on the 97th experimental day. The animal was extremely weak and died the following morning. The average composition of the blood plasma of all the dogs before transfusion was: albumin 1.22 gram per 100 cc., globulin 3.45 grams per 100 cc., and total protein 4.67 grams per 100 cc. The serum used in the transfusions was obtained by bleeding normal dogs. Its average composition was: albumin 3.26 grams per 100 cc., globulin 2.71 grams per 100 cc., and total protein 5.97 grams per 100 cc. The average volume of the transfusions was 288 cc., the average weight of the dogs was 14.8 kilos.

Blood samples were withdrawn by arterial puncture and transferred at once to a 15 cc. rubber-capped graduated centrifuge tube containing sufficient heparin to prevent coagulation. After they had been centrifuged for 20 minutes at 2800 R.P.M., the total volume was noted and most of the plasma removed for chemical analysis. The remaining cells were packed thoroughly by 10 minutes' additional centrifugation at 3200 R.P.M. and the cell volume finally recorded. Percentage cell volume was calculated as 100 times the ratio between cell volume and total volume of the sample. Plasma protein was determined by the method described in a previous paper (1).

Immediate changes resulting from transfusion

To determine the immediate changes resulting from transfusion, samples of blood were withdrawn just before and about 20 minutes after the injection. The transfusions were given through one of the leg veins and occupied 10 to 15 minutes. The change in the plasma protein concentrations in a single experiment is shown graphically in Chart 1. The collective results are listed in Table 1. In every case there was a distinct rise in the albumin concentration, which varied from 0.20 to 0.64 gram per 100 cc., and averaged 0.42 gram per 100 cc. In every case except one there was a distinct lowering of the concentration of globulin, which varied from 0.15 to 0.80 gram per 100 cc., and averaged 0.50 gram per 100 cc. It will be observed that the usual directional change in the

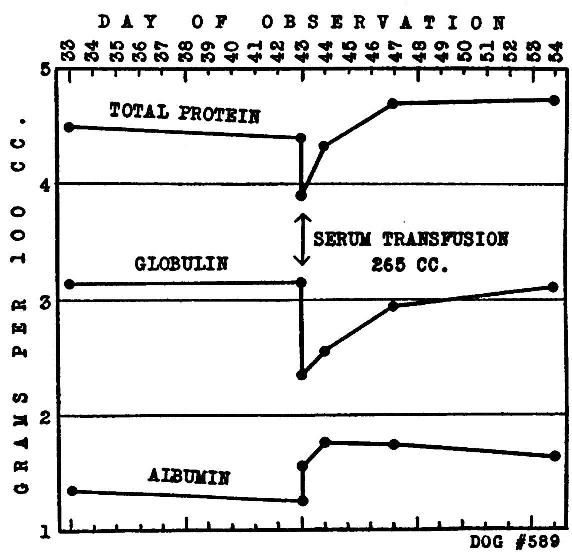

CHART 1. CONCENTRATION CHANGES IN BLOOD PLASMA RESULTING FROM TRANSFUSION WITH 265 CC. OF SERUM

Composition of the serum used was: albumin 4.22 grams per 100 cc., globulin 2.01 grams per 100 cc., and total protein 6.23 grams per 100 cc.

TABLE 1

Plasma concentration changes from serum transfusion

Dog number	Albumin			Globulin		
	Found before transfusion	Found after transfusion	Calculated after transfusion	Found before transfusion	Found after transfusion	Calculated after transfusion
	grams per cent	*grams per cent*	*grams per cent*	*grams per cent*	*grams per cent*	*grams per cent*
448	1.01	1.65	1.61	3.65	3.01	3.37
589a	1.25	1.55	1.68	3.15	2.35	2.34
589b	1.35	1.59	1.73	3.06	2.55	2.89
449a	1.09	1.29	1.35	4.17	3.39	3.65
449b	1.06	1.51	1.28	3.57	3.42	3.32
449c	1.42	1.95	1.68	4.10	3.67	3.45
451	1.36	1.78	1.91	3.05	3.13	3.28
772	1.22	1.79	1.62	2.89	2.67	2.43

protein fractions, namely, an increase in albumin and a decrease in globulin, is the same as that which would have occurred had the recipient's and donor's sera been mixed in vitro. That the changes are more complicated than those involved in a simple mixture becomes apparent when the alterations in the total plasma protein are considered. These are listed in Table 2. Although there are four instances in which transfusion

TABLE 2

Plasma concentration changes from serum transfusion

Dog number	Total protein			
	Before transfusion	After transfusion	Change	Donor's serum
	grams per cent	*grams per cent*	*grams per cent*	*grams per cent*
448	4.66	4.66	0.00	6.77
589a	4.40	3.90	−0.50	6.23
589b	4.41	4.14	−0.27	5.73
449a	5.26	4.68	−0.58	6.01
449b	4.63	4.93	+0.30	6.01
449c	5.52	5.62	+0.10	5.00
451	4.41	4.91	+0.50	6.36
772	4.11	4.46	+0.35	5.64

resulted in an increased total protein concentration, it is seen that on three occasions the concentration was decreased and once remained the same in spite of the fact that the donor's serum contained appreciably more protein than the recipient's. The change in the total protein represents the algebraic sum of the increase in albumin and decrease in globulin and its direction is governed by whichever of the fractions shows the greater change. Moreover, it is clear that other factors participate in producing the final result than those involved in a simple mixture in vivo.

The following possible participating factors have been considered: (1) a portion of the transfused protein may leave the circulation immediately, (2) the transfusion may act as a stimulus so that an additional protein increment is added from the tissues, (3) water may leave the circulation either to be excreted by the kidneys or to enter the tissues, and (4) water may be drawn by osmosis from the tissues into the circulation. An attempt has been made to decide which of these factors is or are most probably concerned in producing the effects observed. The calculations to be outlined involve certain assumptions, the chief of which concerns blood volume, and must, therefore, be regarded as furnishing suggestive evidence only.

Blood volume before transfusion has been calculated on the basis of 100 cc. for each kilogram of body weight. Whipple and his associates (2) using the dye method found the average blood volume of 22 dogs to be 101.3 cc. per kilo. The individual variations were wide and ranged from 83.7 to 115.3 cc. per kilo. McQuarrie and Davis (3), who measured the change in refractivity of serum resulting from the injection of certain colloids, found in 21 dogs an average blood volume of 97.6 cc. per kilo. Individual variations were from 73 to 114 cc. per kilo. These values are in close agreement, although Whipple quotes Gréhant and Quinquaud, who, using the carbon monoxide method, found 82.0 cc. per kilo. The wide normal variations mean that estimations of blood volume on the basis of weight can represent a first approximation only. A recent report by Chang (4) indicates that blood volume is reduced in human nutritional edema. However, because of the malnourished state the volume per kilogram of body weight tends to surpass the usual normal values. On the whole it is believed that the figure assumed for the calculations will err on the side of being greater rather than less than the true volume. The effect of an erroneous assumption on interpretation will be pointed out as the discussion proceeds. The plasma and cell volumes before transfusion have been calculated from the blood volumes and hematocrit readings. The plasma volumes, cell volumes, and total blood volumes after transfusion have been calculated by assuming that the total number of circulating red cells was unchanged by the transfusion. This assumption with the hematocrit readings permits computation of the various volumes. The possibility that splenic contraction or relaxation may have contributed to the shifts in relative cell volume would seem to be excluded by the fact that dog 772 had been splenectomized several weeks before experimentation was begun and that this animal exhibited no reactions at variance with those observed in the other dogs. The observed hematocrit readings before and after transfusion are listed for reference in Table 3.

TABLE 3

Hematocrit readings in relation to serum transfusion

Dog number	Before	20 minutes after	24 hours after	Subsequent
	per cent of cells	*per cent of cells*	*per cent of cells*	*per cent of cells*
448	38.8	29.3	33.4	36.0 (3) *
				36.4 (7)
589a	49.6	37.3	42.0	43.7 (4)
				47.0 (11)
589b	43.0	36.3	35.0	37.8 (5)
449a	40.6	33.1		
449b	25.7	21.5	25.4	
449c	25.4	19.9	26.0	30.2 (4)
				32.5 (20)
451	26.7	24.1		
772	40.6	30.6	31.7	36.0 (4)

* Figures in parenthesis indicate number of days elapsed since transfusion.

Table 4 expresses the changes resulting from transfusion in terms of the total number of grams of protein in circulation, the figures representing the product of plasma volume and concentration. It is seen that the impression, gained from the figures for concentration, of a decrease in globulin is illusory and that actually the circulation contains more globulin as well as more albumin after transfusion than before. Obviously the illusion results from the fact that the plasma volumes were considerably increased. It should be pointed out that whereas the magnitude of the changes shown in Table 4 depends upon the figure assumed for blood volume, nevertheless, the directional change, that is, whether a given fraction is increased or decreased, is independent of this figure.

TABLE 4

Total plasma proteins in circulation

Dog number	Total protein		Albumin		Globulin	
	Before transfusion	After transfusion	Before transfusion	After transfusion	Before transfusion	After transfusion
	grams	*grams*	*grams*	*grams*	*grams*	*grams*
448........	39.1	59.8	8.5	21.2	30.6	38.6
589a.......	32.3	47.3	9.2	18.8	23.1	28.5
589b.......	35.7	44.3	10.9	17.0	24.8	27.3
449a.......	47.7	58.5	9.9	16.1	37.8	42.4
449b.......	52.7	70.7	12.1	21.7	40.6	49.0
449c.......	62.2	86.9	16.0	30.2	46.2	56.7
451........	48.2	61.5	14.9	22.3	33.3	39.2
772........	37.6	63.2	11.2	25.4	26.4	37.8

By taking the sum of the protein fractions present in the circulation before transfusion and the amount of these substances given in the transfusion, it has been possible to calculate the total circulating proteins which should have been found after transfusion if no protein had left the circulation. This figure divided by the second plasma volume gives the concentration which should have been found. The results of such a calculation are given in Table 1. On the whole the agreement between calculated and determined values is rather close in view of the assumptions and limited precision of hematocrit determinations. An average of all figures shows for total protein 4.66 grams per 100 cc. by analysis and 4.62 grams per 100 cc. by calculation. On the other hand it is necessary to state that a similar computation based upon an assumed blood volume of 80 cc. per kilogram yields an average calculated total protein concentration of 5.03 grams per 100 cc. and that this would indicate a loss of 20 per cent of the total amount of transfused protein. It seems safe to conclude, however, that at least the major portion of the injected protein is still in the circulation 20 minutes after the transfusion. It will be shown presently that the circulating proteins slowly decrease during the days following transfusion and it is reasonable to assume that the decrease begins during or immediately after the injection. The very slowness of the subsequent decrease, however, renders rapid removal during the first 20 minutes unlikely and suggests that the figures obtained with an assumed blood volume of 100 cc. per kilogram are more nearly correct than those based on a volume of 80 cc. per kilogram.

TABLE 5

Relation between increase in blood volume and volume of serum transfused

Dog number	Blood volume			Volume of serum transfused
	Before transfusion	After transfusion	Increase	
	cc.	*cc.*	*cc.*	*cc.*
448	1370	1815	445	365
589a	1455	1935	480	265
589b	1420	1680	260	240
449a	1525	1870	345	250
449b	1530	1827	297	225
449c	1510	1930	420	340
451	1490	1651	161	265
772	1540	2042	502	350

Table 5 contrasts the calculated changes in blood volume with the volumes of serum transfused. In seven instances out of eight the former is greater than the latter, a result which suggests that the blood volume increase from transfusion in general is due not only to the actual volume of serum transfused but also in part to an additional increment of water drawn into the circulation from the tissues. Here again, however, the magnitude of the change depends on the figure assumed for blood volume and a computation based on a volume of 80 cc. per kilogram fails to indicate the same degree of blood volume increase. Table 5 shows that dog 451 reacted differently from the other animals, a loss rather than a gain of fluid being suggested. It will be recalled that this dog was practically moribund at the time of transfusion and the different type of reaction can be assumed to have resulted from capillary changes accompanying the physical state. When the result with this animal is excluded the calculation based on the lower blood volume still shows an average increase in blood volume 25 cc. greater than the average volume of serum injected. Such a figure, however, would be too small to permit conclusions. Fortunately, it was possible in another way to look for evidence of additional water being added to the circulation. This was done by comparing relative cell volumes five minutes and twenty minutes after transfusion. In three instances the hematocrit readings during the interval fell 1.0, 1.3 and 1.7 per cent respectively. The changes were not large but it should be noted that large changes could not be expected and the observations were consistently in the same direction. They indicated the addition of 28, 33 and 58 cc. of water to each liter of circulating blood. When it is recalled that the blood volume adjustment must begin during the process of transfusion and that measuring the change between 5 and 20 minutes after the injection cannot record the full effect, these figures are seen to be in rough agreement with those in Table 5. They, therefore, suggest that 100 cc. per kilogram is a closer approximation of blood volume than 80 cc. per kilogram.

A review of the preceding paragraphs leads to the impression that the deviation in vivo from what would be expected if donor's and recipient's sera were mixed in vitro results in part from the loss of a small amount of protein from the circulation and in part from an additional fluid increment drawn into the circulation from the tissues. The magnitude of the respective changes undoubtedly varies in individual cases.

A word should be added concerning physical reaction to the transfusions. Unfortunately, no record was kept of respiration, pulse and temperature changes. In six instances out of the seven, however, in which the dogs survived, the only apparent untoward effect was a variable diminution in appetite which lasted for several days. In the seventh instance (589a) the reaction was profound. Before the injection of 265 cc. of serum had been completed, saliva began to drool from the mouth and the dog vomited bile-stained fluid. This was followed by a large loose evacuation of the bowels. After the operation the animal was prostrated, lay without moving for 15 to 20 minutes and exhibited a rapid thready pulse. Recovery was gradual but by the following morning the animal appeared normal and had eaten all of the diet offered the previous day. The same dog, when transfused again 15 days later, showed no untoward reaction.

Subsequent changes resulting from transfusion

The data now at hand for studying the serum changes occurring through subsequent days following a transfusion are more meager than those available for the immediate alterations. We have already stated that dog 451 died and that the first transfusion with dog 449 was followed by hemorrhage. Six observations, therefore, remain on the state of the blood at the end of 24 hours. The three transfusions with dog 449 were given on consecutive days and the later changes must be considered separately. With the four remaining transfusions observations extended over 4, 5, 7 and 11 days.

One example of the later concentration changes has been given in Chart 1. At the end of 24 hours the albumin concentration had further increased in three instances and decreased slightly in three whereas the globulin concentration after its immediate fall always tended to rise again. This delayed rise in the albumin was in neither instance as great as the immediate. In one case a still further slight increase was noted after 3 days but in every instance within a few days the level began to decline slowly. In no case, however, within the observation period did it reach that present before transfusion. In fact, the rate of decline has not been greater than might have taken place as a result of the dietary deficiency alone. As a result of two transfusions the concentration of albumin in dog 449's serum was raised from 1.06 to 1.95 gram per 100 cc.; it fell rapidly during the next 6 days to 1.32 gram per 100 cc. and afterwards declined very slowly, the level on the 27th day following transfusion being 1.22 gram per 100 cc. In general the plasma globulin also tended to rise for several days, subsequently either maintaining the level reached or showing a gradual decline.

Expression of the subsequent changes in terms of total circulating proteins has presented even greater difficulties than those previously outlined. In no instance has the relative cell volume (hematocrit reading) returned to a figure as high as that present before transfusion (Table 3). Whether this fact is to be explained on the basis of a slowly developing anemia or due to a slight persistent increase in plasma volume, is not known. For the present purpose we have assumed the latter, as the assumption tends to minimize the magnitude of the effect to be observed, and have calculated subsequent blood and plasma volumes on the basis of hematocrit readings and the initial cell volume. The results suggest a gradual decline in blood volume and in circulating amounts of both albumin and globulin toward the values existing before transfusion. Tables 6 and 7 furnish two examples of this decline. There are several

TABLE 6

Proteins retained in circulation after transfusion
Dog 448

Time after transfusion	Albumin	Globulin	Total protein	Blood volume
	grams	*grams*	*grams*	*cc.*
Before	8.5	30.6	39.1	1370
20 minutes	21.2	38.6	59.8	1815
1 day	19.4	33.5	52.9	1716
3 days	19.2	32.9	52.1	1592
7 days	17.8	30.4	48.2	1477

TABLE 7

Proteins retained in circulation after transfusion
Dog 589a

Time after transfusion	Albumin	Globulin	Total protein	Blood volume
	grams	*grams*	*grams*	*cc.*
Before	9.2	23.1	32.3	1455
20 minutes	18.8	28.5	47.3	1935
1 day	17.6	25.5	43.1	1719
4 days	16.3	27.4	43.7	1652
11 days	13.3	25.2	38.5	1536

discrepancies, probably due to analytical error, but the general trend is seen definitely to be as outlined. In the case of the dog which received the consecutive transfusions the quantitative declines were exaggerated. Figures for nitrogen balance are available for most of the dogs of the series. They have failed to indicate any unusual variation in the rate of nitrogen elimination either on the day of transfusion or during subsequent days. The finding is in accord with the theory that the circulating protein is gradually removed; but, because the quantities of nitrogen involved are small, it gives no evidence concerning disposition of the protein withdrawn from the blood.

A final word must be added concerning the effect of the transfusions on the edematous state. We had hoped originally to produce serum albumin elevations great enough to bring about diuresis and disappearance of edema. This object was not attained. In several instances the amount of edema, if changed at all, was increased rather than decreased 24 hours after the transfusion. Body weight failed to supply interpretable data. In two instances the weight increased by several tenths of a kilogram the day after transfusion and then declined to a new low level, but such fluctuations were observed often enough without transfusion. Dog 449 lost 2.5 kilograms during the two weeks following her consecutive transfusions; but, as there was no clinical evidence of decrease in edema, the loss seemed more related to a general debilitated condition with loss of appetite than to water excretion.

Hartmann and Senn (5) have recently described nephrotic patients in whom diuresis and loss of edema occurred when the plasma oncotic pressure was raised by intravenous injections of gum acacia. The reason for failure to obtain a similar effect by serum transfusions in edematous dogs is not known. It is possible that the rapid increase in blood volume which accompanies the procedure may be associated with an increase in blood pressure within the capillaries which offsets the heightened oncotic pressure. The work of Chang (4), however, suggests that blood volume increase may be a normal accompaniment of increased plasma protein and it is difficult to believe that an increase of this nature should be associated with unusual mechanical pressure within the capillaries. It must also be remembered that with the amounts of serum transfused the plasma albumin concentrations were not raised to anything approximating normal values and it is possible that with larger transfusions or preferably by the use of more concentrated sera, a more definite effect on the edematous state would be observed.

SUMMARY

1. Experiments were performed on dogs, exhibiting serum albumin deficits produced by protein starvation, which indicate that transfusion with normal dog serum is followed by an immediate rise in the concen-

tration of serum albumin and usually by a fall in the concentration of plasma globulin. When the results are expressed as total circulating protein, rather than in terms of concentration, it is seen that both albumin and globulin are increased by the procedure.

2. It is shown that the results obtained are not to be explained entirely on the basis of a simple mixture of donor's and recipient's sera in vivo. By means of calculations, which depend upon an assumed figure for blood volume, it is shown that two possible participating factors may be (*a*) a loss from the circulation of a small amount of the injected protein and (*b*) an additional increment of water drawn into the circulation from the tissues. Because of the assumptions the evidence is regarded as suggestive only.

3. In the days following transfusion it is shown that the albumin concentration, after first rising, slowly declines; although it does not, within a period of a week or two, return to the level present before transfusion. Globulin concentration, after an immediate fall, rises rapidly for several days and then either remains at or slowly recedes from the level reached. When the results are expressed in terms of total circulating proteins, it appears that the quantities of albumin and globulin in the circulation as well as the blood volume decline gradually during the subsequent days in the direction of the values present before transfusion.

4. Significant alterations in the edematous state did not occur as a result of the transfusions.

BIBLIOGRAPHY

1. Weech, A. A., Snelling, C. E., and Goettsch, E., J. Clin. Invest., 1933, xii, 193. The Relation between Plasma Protein Content, Plasma Specific Gravity and Edema in Dogs Maintained on a Protein Inadequate Diet and in Dogs Rendered Edematous by Plasmapheresis.
2. Hooper, C. W., Smith, H. P., Belt, A. E., and Whipple, G. H., Am. J. Physiol., 1920, li, 205. Blood Volume Studies. I. Experimental Control of a Dye Blood Volume Method.
3. McQuarrie, I., and Davis, N. C., Am. J. Physiol., 1920, li, 257. Blood Volume Studies. IV. Blood Volume as Determined by the Change in Refractivity of the Serum Nonprotein Fraction after Injection of Certain Colloids into the Circulation.
4. Chang, H. C., Proc. Soc. Exper. Biol. and Med., 1932, xxix, 829. Plasma Protein and Blood Volume.
5. Hartmann, A. F., and Senn, M. J. E., (Data reported at meeting of American Pediatric Society, Rochester, Minn., May 27, 1932), Am. J. Dis. Child., 1932, xliv, 673. Studies in Edema with Particular Reference to the Therapeutic Value of Acacia.