ANNALS OF SURGERY

VOL. 128 DECEMBER, 1948 No. 6

THE NATURE OF THE SHIFT OF PLASMA PROTEIN TO THE EXTRAVASCULAR SPACE FOLLOWING THERMAL TRAUMA*†

Oliver Cope, M.D., John B. Graham, M.D., Francis D. Moore, M.D. and Margaret R. Ball, A.B.

Boston, Mass.

FROM THE SURGICAL RESEARCH LABORATORIES OF THE HARVARD MEDICAL SCHOOL AND THE SURGICAL SERVICES AT THE MASSACHUSETTS GENERAL HOSPITAL, BOSTON, MASS.

Discussion of the circulatory disorder of the burned patient and of the need for fluid to survive is inadequate if it does not include a consideration of the abnormal distribution of the plasma proteins occurring within the body as the direct result of the injury. In the burned, as in the healthy, it is the colloid osmotic pressure exerted by the plasma proteins in differential concentrations on either side of the semi-permeable capillary membrane which apportions water between plasma and interstitial space and maintains blood pressure. It is the sudden increase in permeability of the capillary caused by the burn which destroys the osmotic pressure of the plasma in the area of injury and permits the collection of edema in the wound.

Two misconceptions regarding the distribution of plasma protein are current. First, it is believed that the proteins of plasma circulate only within the blood stream. On the contrary, the normal capillary membrane is incompletely restrictive; albumin, and to a lesser extent globulin, pass out in small concentration from the plasma into the interstitial fluid. The experiments of Drinker on the lymph of dogs, and of others, have made this clear.[1] The lymph protein, and therefore presumably that of the interstitial fluid, varies from less than 1 per cent in the superficial tissues of an extremity to as much as 5.0 per cent in the liver. Though data are limited in the human being to thoracic duct lymph and various edema fluids, it is reasonable to expect that comparable quantities of protein will be found in man outside of the blood vessels. Since

* Submitted for publication November, 1947.

† The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

This study was also aided by a grant from the Josiah Macy, Jr., Foundation.

This work was also aided by a grant from the Ciba Pharmaceutical Products, Inc., Summit, N. J.

the protein outside the vessels is still in circulation, eventually reentering the venous blood via the lymph channels, such protein must be taken into account when estimating protein requirements and water distribution in the burned patient.

The second misconception deals with the change in concentration of protein in the plasma following the burn. Because plasma protein has been proved to leak out of the capillary into the wound it has been stated repeatedly that the remaining plasma has a lowered protein concentration. The reverse is true. The lowered protein concentration found some hours after injury is a secondary phenomenon, the result either of withdrawal of water from unburned tissues, or therapy, or of both.

Because an understanding of the redistribution of water and electrolytes is essential in the care of the burned patient, an account is given of the observations, both experimental and clinical, made by the group studying burns at the Massachusetts General Hospital, bearing on the nature of the shift of protein following a burn.

EXPERIMENTAL OBSERVATIONS

The disordered distribution of protein as a result of a burn has been approached experimentally by collecting lymph flowing from the burned foot of the dog. The abnormal flow of lymph engendered by the burn indicates the volume of water passing through the wound, while the discrepancy in protein between the circulating plasma and lymph indicates the permeability of the capillary to protein. The nature of the water and protein leakage from the plasma and the effect of the leakage upon the residual plasma have been examined. Abnormal proteins have also been searched for.

Lymph Flow and Protein Leakage. The experimental method used is that of Drinker. Field, Drinker and White in 1932[2] described an increased flow of water and protein from capillary to lymphatic trunk through an area of sterile inflammation induced by a burn. The foot of a dog was burned by immersion in hot water. The lymph was collected through a cannula placed in a lymphatic trunk above the ankle. Following the injury the protein concentration rose from the normal of 2 Gm./100 cc. to nearly 4 Gm. The flow initially increased by the heat, returned after two and a half hours to that before injury. This classic observation, by measurement of lymph flow and protein concentration, of abnormal capillary filtration in a burn wound of the foot of the dog has been amply confirmed in further work by Drinker and his collaborators,[3] and by ourselves.[4] Evidence of increased flow in the lymphatics in burns has also been obtained by McMaster in smaller animals.[5, 6]

The degree of capillary damage depends to an extent upon the intensity of the burn.[7] A burn of the dog's foot by water at 67 degrees C. for 10 seconds is a threshold burn; it produces a slight, transient increase in lymph flow from that foot and a small but significant rise in concentration of the lymph protein. More intense burns, by hotter water and by longer immersion, are followed by greater flow and protein concentrations; but no matter how severe the burn, the protein concentration of the lymph is never as high as that of plasma. A cooking burn of boiling water for 20 seconds resulted in a lymph protein concentration of only 5.0 Gm. while the plasma protein was 6.7 Gm. The findings in a typical experiment of the dog's foot with a burn of moderate severity are depicted in Chart 1; the protein concentration of the lymph rose after burning from 1.5 Gm. to 3.5 Gm./100 cc. (See also Table I; compare slight rise in protein concentration of lymph from left foot with that from more severely

TABLE I.—*Distribution of Albumin and Globulin Proteins in Arterial Plasma and Lymph from the Foot of Dogs Before and After Burning.*

Sample		Time Hours	Arterial Pressure mm. Hg.	Hema-tocrit % Cells	Protein Concentrations: Total Gm./100 cc.	Albumin Gm./100 cc.	Globulin Gm./100 cc.	AG Ratio	NPN mg./100 cc.
Experiment No. 1									
Plasma	Pre-burn.........	..	131	44	5.9	4.4	1.5	3.0	23
	Post-burn........	1	144	55	6.4	3.9	2.5	1.6	21
	Post-burn........	2	...	62	6.1	3.7	2.4	1.5	22
	Post-burn........	3½	143	68	5.9	3.5	2.4	1.5	31
Lymph	Pre-burn.........	...	...	..	0.9	0.7	0.2	3.5	31
	Post-burn........	1	...	..	3.3	2.3	1.0	2.3	28
	Post-burn........	3	...	..	4.1	...	...	...	27
	Post-burn........	4	...	..	4.4	2.6	1.9	1.4	30
Experiment No. 2									
Plasma	Pre-burn.........	...	...	34	6.7	3.4	3.3	1.0	45
	Post-burn........	4	...	32	6.6	3.4	3.2	1.0	83
Lymph	Pre-burn......... (Both feet)	...	...	..	2.2	1.1	1.1	1.0	..
	Post-threshold burn (Left foot)	4	...	..	2.7	1.3	1.4	0.9	..
	Post-severe burn...	2	...	..	4.5	2.9	1.6	1.8	..
	Post-severe burn... (Right foot)	4	...	..	4.5	2.6	1.9	1.4	..

burned right foot.) It is thus clear that a portion only of the plasma protein passes with the water through the damaged capillary from blood vessel into the lymphatic; there is an unaccounted for fraction, 1.7 Gm. in the cooking burn described and more in less intense burns.

Where is this unaccounted for fraction of the protein? That it does not lodge in the wound is indicated by the observation that fluids removed by needle from the wound and exuding on the surface of the wound have protein concentrations slightly lower than that of the lymph.[8] There is only one place for it to be and that is still in the blood stream.

Protein Concentration of the Residual Plasma. If the unaccounted for plasma protein remains in the blood stream, dissolved in other plasma water, then the concentration of the protein in the residual circulating plasma must rise, not fall, immediately following the thermal injury. In support of this assumption is the finding of an increased protein concentration of the plasma of dogs after burning. Plasma protein concentration was followed in 48 dogs burned experimentally (under nembutal anesthesia) and receiving no fluid therapy. In 38 an increase in the protein concentration was observed. In 23 of the 38, only one or two feet were burned and the protein elevation ranged from 0.1 to 1.3 Gm./100 cc., with an average of 0.44 Gm.; in three of these a subsequent dilution was noted, early in one (Experiment 1 of Table I) and late in two. In the other 15 a larger surface of four legs was burned and the protein concentration rose from 0.1 to 4.7 Gm., averaging a 2.09 Gm. rise. Of the 10 remaining animals of the 48, in six, no significant change in concentration was recorded, and in four, falls in concentration of 0.2, 0.3, 0.8 and 1.3 Gm./100 cc. were found. However, the first reading after the burning in each of the 10 animals was delayed, being respectively at the sixth, eighth, 28th and 19th hours in the four whose concentration fell. It is more than likely that a period of increased concentration was overlooked. (Of three dogs maintained under nembutal anesthesia but not burned, two showed a 0.2 Gm. decline and the third a 0.2 Gm. increase in the protein concentration; all three showed a slow rise in hematocrit.)

Osmotic Tension of the Residual Plasma. The increased concentration of protein found in the residual circulating plasma means an increase in the osmotic tension of the plasma which should be accompanied by a withdrawal of fluid from the unburned tissues of the body to the blood stream. The dilution of protein succeeding the initial increased concentration indicates such withdrawal (Experiment 1, Table I).

What evidence is there that the initially more concentrated plasma does not also take back water from the wound? The finding of the slightly lower protein concentration in wound and exudate fluid than in the lymph suggests resorption of water from the edema fluid before it enters the collecting lymphatic trunk. That such resorption by plasma of water in the wound is limited, however, is indicated by the following experiment, using hypertonic purified albumin.* The lymphatic trunks of the hind feet, the neck and the thoracic duct were cannulated; a control flow of lymph was observed. The feet were then burned and when the typical curve of increasing lymph flow from the feet and rising serum protein concentration and hematocrit of the blood were established, the animal was given intravenously 31 Gm. of the albumin in a 25 per cent solution. The hematocrit fell promptly, reaching its lowest level two hours after the injection. This fall in hematocrit represented an enlargement of the plasma volume due to absorption of fluid from the tissues. That this fluid came virtually entirely from the unburned tissues and not from the burn wounds is indicated by cessation of the flow of the lymph from the cervical trunk and a drop in thoracic duct pressure while the increased flow from the feet engendered by the burn continued. There was a slight decrease in flow of the lymph from both feet in the first 20 minutes after the injection

* This experiment was carried out in collaboration with Dr. James T. Heyl. We are indebted to him for his help and to Professor Edwin J. Cohn for furnishing us with the purified bovine albumin used.

followed promptly by a secondary rise to a rate of flow as high as that before the injection. This transitory decline in flow was no greater than that often encountered in control experiments, but because it appeared simultaneously in both feet is believed to indicate a transitory drop in tissue pressure presumably due either to a decrease in rate of capillary filtration into the wound or to an increase in rate of water resorption from the wound area.

The cessation of flow of the lymph from the cervical region persisted for more than three hours in spite of massage and indicated severe dehydration of this unburned region of the body. The contrast between this cessation and the transitory decrease in lymph flow from the wounds suggests that the mechanism for resorption of water locally is also damaged by a burn, the only mechanism for return of fluid from the wound remaining intact being the lymphatic system.

The osmotic influence of the increased concentration of protein found in the circulating plasma (immediately after injury) upon the water concentration of the unburned tissues should depend in part upon its albumin globulin ratio. The lower the ratio the less dehydrating it should be. In the next section the differential permeability of the damaged capillary membrane to albumin and globulin and its effect upon the albumin globulin ratio of the residual plasma is, therefore, considered.

Permeability of Capillary to Albumin and Globulin. Perlmann, Glenn and Kaufman in 1943 examined by electrophoresis the serum and lymph of calves before and after burning.[9] In keeping with the earlier chemical analyses on dogs, as well as on calves, the serum proteins, albumin, alpha, beta and gamma globulins, are to be found in normal lymph; the relative amount of albumin in lymph is higher than in the serum. When first examined, two hours or longer following thermal injury in the calf, the albumin globulin ratio of both serum and lymph had fallen; a greater fall was encountered in the lymph from the burned than unburned leg. Perlmann *et al* concluded that a primary effect of the injury on the capillary wall was "the increased passage of the plasma proteins and a decrease in the differential permeability to albumin."

The permeability of the damaged capillary has been investigated in this laboratory with radioactive diazo dyes and by chemical analysis of the proteins in both the plasma and lymph of dogs. The experiments with the radioactive dyes have been published elsewhere.[4] The dyes, made radioactive by the chemical addition of radioactive bromine, have a rapid and preferential affinity for albumin.[10]* When injected intravenously in less than saturating quantities they bind with the plasma albumin. The rate of their subsequent appearance in lymph is a measure of the rate of passage of albumin across the capillary membrane. Following a severe burn, immersion in boiling water for 60 seconds, the dye concentration in the lymph flowing from the burned foot

* The addition of two bromine atoms to the lipoid soluble portion of the dye molecule apparently decreases water solubility; the colloidal property of the dye and its "fastness" as a dyestuff as well as its biologic properties relative to the protein bond are apparently unchanged.[11]

rose to that in the blood serum within less than an hour. The total protein concentration had risen in the lymph from 2.0 Gm. to only 4.3 Gm./100 cc. while in the serum it started at 7.1 Gm. and gradually rose to 7.7 Gm./100 cc. The conclusion from this experiment was that the capillary wall had become, as the result of the burn, wholly permeable to albumin but not to the globulins.

After a less severe burn, boiling water for only 15 seconds, the dye concentration took longer to rise in the lymph and never reached that of the serum, indicating a still incomplete though increased permeability to albumin.

The chemical analyses** of the plasma and lymph proteins of dogs carried out in this laboratory support the concept of a predominant loss of albumin through the damaged as well as normal capillary membrane. The plasma proteins were examined in 11 experiments. In seven the alubumin-globulin ratio fell, the minimum decrease being from 0.7 to 0.6 and the maximum from 1.5 to 0.6. These seven experiments include the three in which the ratio was measured at two hours or less after injury. Experiment 1 of Table I is one of these three; the pre-burn ratio was unusually high. In two experiments the ratio rose, from 1.0 before burning to 1.6 at the fifth hour and from 2.5 to 3.5 at the 28th hour, respectively, after burning. In the remaining two experiments the ratio did not change, including Experiment 2 of Table I in which the ratio was not measured within the first four hours after burning.

In only four of these 11 experiments was there sufficient lymph for chemical determination of the ratio both before and after burning. In two there was a rise in the ratio. This rise is illustrated in the lymph from the severely burned foot (right) of Experiment 2, Table I, the ratio being greater at two hours than four hours post-burn. There was no significant change in the ratio in this same experiment (Experiment 2, Table I) in the lymph from the lightly burned left foot; the total protein rose only 0.5 Gm. In the other two experiments, there was a fall in the ratio, but in both experiments the initial ratio of plasma and lymph was unusually high (see Experiment 1, Table I).

The fall of the albumin-globulin ratio of the plasma encountered immediately after injury should reduce the rise in osmotic tension indicated by the increase in concentration of the total protein.

Abnormal Proteins. Perlmann, Glenn and Kaufman in their electrophoretic studies in the calf discovered in the lymph from the burned leg a hitherto unseen protein in the range of globulin mobility. They conclude that the substance is a protein released from damaged cells. Our efforts toward identification of abnormal proteins were limited to a study of the activity of certain enzyme systems. Zamecnik[13] has been able to recover in the lymph from the burned feet of dogs specific peptidases of the types associated with intracellular enzyme systems; it is presumed, therefore, that they were released from the cells by the damage.

The amylase and cholinesterase activities of serum and lymph before and after burning have also been examined. The activities found in seven dogs,

** Chemical separation of the plasma and lymph proteins was carried out by the sodium sulfate method of Howe.[12]

both in serum and lymph, were proportional to the total protein concentrations. The slight variations in activity were no greater than those encountered in control animals. The variations usually, but not always, paralleled alterations in the protein concentration. These findings are interpreted to mean that there is no specific effect of burn trauma either upon the activity of the two enzyme systems as they relate to the extracellular fluid (plasma and interstitial fluid), or upon the permeability of the capillary membrane creating a predilection for their passage.

The fibrinogen level* of blood and lymph was followed in four dogs before and after burning. The normal level of fibrinogen in the lymph was less than half that in the plasma. Following burning the level in both plasma and lymph rose, slightly in the plasma, more in the lymph until that of the lymph was approximately that of the plasma. (There was thus no evidence obtained in these few experiments of retention of fibrin in the wound.)

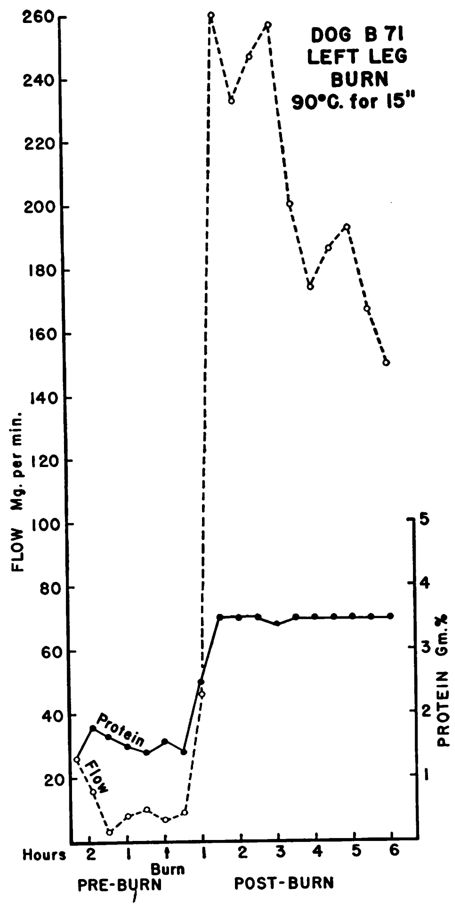

CHART I.—The increase in flow and protein concentration of lymph from the foot of a dog following burning. The flow of lymph before burning was obtained only with massage; following the burn there was a spontaneous flow the rate of which rose precipitously in the first hour and a half. In the same period the protein concentration more than doubled. After maximum edema had been reached the flow gradually decreased but the protein concentration remained at the same elevated level.

CLINICAL OBSERVATIONS

It has not been feasible to examine the lymph of burned patients; analysis of the changes in capillary permeability and the shift of protein to the wound, therefore, must depend upon a comparison of blood plasma and the fluid removed from the blebs of the wound.

The chemical and electrophoretic studies of plasma and bleb fluid carried out on this series of burned patients confirm those on animals. As the plasma circulating through the wound area loses water into the wound, a portion of the proteins dissolved in that water is retained within the vascular tree, and there is conse-

* Fibrinogen of plasma and lymph was measured by the method of Cullen and Van Slyke.[14] The normal plasma level is from 0.3 to 0.6 Gm./100 cc.

quently an initial rise in plasma protein concentration and in osmotic power.

Contrast of Plasma and Bleb Fluid Proteins. The literature records several measurements of the protein withdrawn from the blebs of burned patients;[15, 16] the concentrations recorded are 60 to 80 per cent of that expected of the plasma. The interval between injury and withdrawal of the fluid and a simultaneous plasma protein measurement are usually not also recorded.

The total protein concentration of the fluid of a number of unruptured blebs was examined at varying intervals following injury. The fluid was withdrawn, without breaking the bleb, by sterile needle and syringe inserted at the side or base of the bleb. A few of the patients afforded a sufficient number of blebs for repeated taps. A fluid was discarded if the gross appearance of the wound suggested infection or if the fluid was cloudy on withdrawal. Most of the fluids were cultured to insure exclusion if bacterial inflammation were present. The concentration of the plasma was measured simultaneously in most instances. The rate of passage of sulfadiazine from blood stream to bleb fluid was measured in three patients at intervals after injury (2, 60 and 100 hours) in order to ascertain whether the intimacy of bleb fluid and blood plasma persisted.

TABLE II.—*Concentrations of Plasma and Bleb Fluid Proteins of Burned Patients at Varying Intervals Following Burning.*

		Bleb Fluid Proteins				
Case No.	Time Post-Burn	Total Gm./100 cc.	Albumin Gm./100 cc.	Globulin Gm./100 cc.	AG Ratio	Plasma Protein Gm./100 cc.
116	2 hrs.	4.2	See Table III		1.71	7.2
63	2 hrs.	4.6				
58	3 hrs.	5.1	3.9	1.2	3.2	
114	36 hr .	3.1	...	...	...	5.7
120	51 hrs.	4.3				
120	54 hrs.	4.7	...	...	...	6.5
81	3 days	4.4	2.8	1.6	1.8	
80	5 days	4.9	2.9	2.0	1.5	
119	5 days	3.6	...	...	...	6.6
119	5 days	3.2	...	...	...	6.5
119	6 days	4.0	...	...	...	6.5
28	5 days	3.6	2.1	1.5	1.4	6.1
28	6 days	3.3	...	...	...	5.9
28	12 days	2.5				
17	6 days	2.2	...	...	...	8.1
38	7 days	1.5	...	...	...	5.4
19	7 days	1.8	...	...	...	7.0

The total protein concentration of the fluid removed from a bleb of a burn wound was found to be always lower than that of the patient's circulating blood plasma. The chemical analyses from representative patients with partial thickness burns are given in Table II. The analysis of the electrophoretic pattern of the fluid and plasma of one of two patients, generously examined for us by Dr. G. E. Perlmann, is given in Table III. The protein concentrations of bleb fluid and plasma of patients correspond closely to those of the lymph from the burned foot and plasma of dogs. The highest concentrations in the bleb fluid are found within the first days following the burn. The fluids withdrawn after the fifth day show concentrations decreasing with time, suggesting not only resorption or breakdown of proteins as healing progresses, but also that the protein concentration of the normal interstitial fluid is considerably lower than that of the wound edema fluid, and at least as low as that of the normal peripheral lymph of the dog.

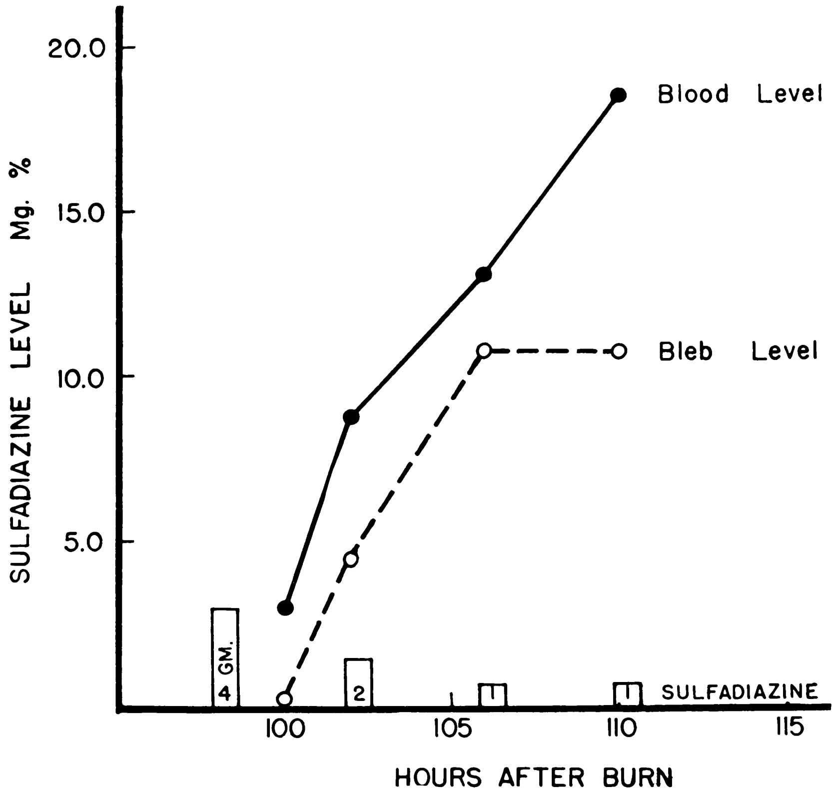

CHART II.—Case 119. Concentration of sulfadiazine in blood and bleb fluid after withholding the drug until the fourth day. The patient had a partial thickness skin burn with multiple blebs. Four grams of sulfadiazine were given by mouth 98 hours after the burn. The rise in concentration in the bleb fluid is almost as prompt as in the blood stream indicating rapid exchange through the wound even at this late time after the burn. (Reprinted with the permission of The Journal of the American Medical Association.)

The albumin-globulin ratio of bleb fluid is high, higher than that of the plasma in the first hours after injury (see Table II, Case 58, and Table III). The observations made after 36 hours show it to be in the range of that of normal plasma. Too few observations have been made to tell when the ratios of plasma and bleb fluid approach each other. The ratio of the one bleb fluid observed at 36 hours was still increased as compared with that of the plasma, but the plasma had an abnormally low ratio and total protein concentration, perhaps related to the patient's preexisting, diffuse osteoporotic disease of the bones of unknown etiology.

The results of the study of the passage of sulfadiazine from plasma to bleb fluid have already been reported[17, 18] but a resumé is given here to emphasize the rapidity with which the plasma and the fluid of the non-infected burn bleb communicate with each other, even as long as five days after injury. To a patient with several blebs suitable for multiple tappings, sulfadiazine was given by mouth at the ninety-eighth hour after burning (Chart II). The level of sulfadiazine was observed in both plasma and bleb fluid by analysis of samples of each fluid taken at the second, fourth, eighth and twelfth hours after the first dose of sulfadiazine. The rise in the level of sulfadiazine in the bleb fluid was found to lag only slightly behind that in the plasma.

These observations in burned patients point to an increased but incomplete permeability to protein of the capillary membrane damaged by a burn, as in the dog. There is apparently a greater permeability to albumin immediately following injury. That the findings in the bleb fluid are not due to a stagnant puddling of the fluid is suggested by the promptness of the passage of the sulfadiazine from the plasma into the bleb.

TABLE III.—*Electrophoretic Observations on Blood Serum and Bleb Fluid of Case 116. (Electrophoresis carried out in Na-diethylbarbiturate buffer of pH 8.6 and ionic strength 0.1)*

			Concentrations in Percent of Total Protein				
				Globulin			
Time Post-Burn	Protein Gm./100 cc.	AG Ratio	Albumin	Alpha-1	Alpha-2	Beta	Gamma
Serum No. 1—2 hours....	7.2	1.00	50.0	7.9	6.4	18.9	16.5
Serum No. 2—6 hours....	5.5	.85	45.9	10.1	11.3	17.8	14.9
Bleb fluid—2 hours......	4.2	1.71	63.1	7.4	3.7	12.3	13.5

Protein Concentration of Residual Plasma. If one can assume that bleb fluid is essentially burn wound fluid, the filtrate from the damaged capillaries, the finding that the bleb fluid protein concentration is lower than that of the plasma, indicates that only a part of the plasma protein escapes with the plasma water from the capillary into the wound.* In the patient as in the experimental

* In the burn wound both of the human being and of the dog there are doubtless gradations of capillary damage and of increased permeability of the capillary membrane. The capillaries nearest the surface must receive the maximum trauma and their membranes may be rendered so permeable that circulation ceases within them, the lumen becoming plugged with cells. Those deepest in the wound must receive minimal damage and, as judged by the experimental observations,[7] allow to seep out a fluid only slightly increased in volume and with a protein concentration only slightly higher than normal. From the capillaries between these extremes, the volume and the protein concentration of the filtrate presumably varies with the depth of the capillary. Thus the wound fluid, or the lymph flowing from a wound, should be a mixture of capillary filtrates. Whether or not the fluid is a mixture is an academic question; the concentration of protein remaining in the vascular tree will depend upon the final concentration in the entire wound area and the total volume of fluid seeping into the wound rather than upon the concentration of the filtrate from any one capillary.

animal, therefore, an increase in concentration of the protein in the residual circulating plasma is to be expected in the first hours after injury. It is of course not possible to obtain in the burned patient the necessary control blood sample to test this assumption, for the patient arrives in the hospital already burned. Based on two sets of observations, we have the impression, however, that such is the case.

The protein concentration of the plasma within the first three hours after injury and before the onset of therapy in 19 extensively burned patients in whom this measurement was obtained, was 7.0 Gm./100 cc., the range being from 6.3 to 7.7 Gm. The lowest level encountered was in the plasma of a six-year-old boy who had long been a feeding problem and was undernourished; also included are another previously ill patient and three chronic alcoholics, all of whose levels were among the lowest found. The average of the highest protein concentration observed within the first 12 hours after injury and after onset of therapy in 46 patients with either extensive burns or circumscribed

TABLE IV.—*Plasma Protein Concentration of Two Extensively Burned Patients in the Initial Hours after Injury.*

Case No.	Extent of Burns	Hours After Burning	Plasma Protein Gm./100 cc.	Hematocrit % Cells
217	78%	0.8	6.9	50
		1.5	6.5	43
		5.5	6.9	50
		8.0	8.0	51
		11.0	7.3	48
		17.0	6.0	39
		20.0	5.4	42
254	45%	1.3	7.7	50
		3.5	8.0	50
		4.5	7.1	47
		8.0	7.4	46
		11.0	7.4	47
		14.5	6.4	43

skin burns and pulmonary damage, was 7.5 Gm./100 cc., with a range of 6.3 to 9.5 Gm. These averages and ranges are above those generally accepted as normal. Phillips, Van Slyke *et al*[19] give a range of 6.0 to 7.3 Gm./100 cc. Our own observations agree with this range for normal adult human beings.

The reinforcement of the concept that the initial change in plasma protein level is toward an increasing concentration was encountered in burned patients whose therapy was either delayed or adjudged inadequate in the first few hours after entry to the hospital. The findings in two patients (Cases 217 and 254) illustrating this event are given in Table IV. During the period of inadequate treatment there was either no change or a rise in the total protein concentration of the plasma. Inadequacy of therapy in these patients was judged by the failure of the hematocrit to fall promptly toward normal, or because it rose further, or because the urinary output was meager. In contrast, the extensively burned patients receiving therapy adequate for rehydration as judged by a falling hematocrit and renal output of 30 cc. or more per hour, were found to show a decrease in plasma protein concentration.

COMMENT

Survival of the burned patient depends upon the maintenance of a physiologic environment for the cells of the unburned organs. Excessive dehydration or hydration can conceivably result in irreversible injury. From examining the shift in protein from capillary to wound, the character of the residual protein in the plasma has been determined; it is this protein which controls the osmotic balance between plasma and the interstitial fluids caring for the nutrition of the unburned cells. Its initial increase in concentration in the absence of therapy means that the unburned tissues are being dehydrated.

Two qualifications are indicated in interpreting the observations recorded in this paper. The osmotic tension of the residual circulating plasma is presumably not as high as suggested by the total protein concentration; the decrease in the albumin-globulin ratio tends to cancel the rise in osmotic power. No measurements of the osmotic tension were carried out.

The second qualification is in regard to the origin of the decrease in the albumin-globulin ratio in the plasma. In addition to the preferential loss of albumin through the damaged capillary into the wound, new globulin may be added to the circulating plasma. White and Dougherty[20] have described in experimental animals a disintegration of lymphocytes with release into the circulation of globulin protein as a result of the administration of either adrenotropic or adrenocortical extracts. Selye has found adrenotropic and adrenocortical hyperactivity to be an expected sequel of trauma.[21] The relation of the lymphocyte-protein release to the endocrine system in the human being following burn trauma is discussed in a subsequent paper.[22] But it remains to be discovered to what extent in the burned patient such a reaction to trauma contributes to the change in albumin-globulin ratio of the plasma of the burned patient. It obviously cannot account for the entire fall of the ratio in the plasma; in such case the ratio in the lymph of the burned dogs and in the bleb fluid of the patients would be identical with that of the plasma.

The seeming difference between the observations of Perlmann, Glenn and Kaufman in the burned calf and ours in the burned dog and human being is probably one of interpretation, not of fact. It is possible that had Perlmann et al measured samples of lymph within the first two hours after injury, results comparable to ours in the same period would have been found.

The interest of Perlmann and her colleagues was centered on the search for abnormal proteins, the result of burn damage; a hitherto unidentified protein in the range of globulin mobility was disclosed by electrophoresis in the burned calf. Doctor Perlmann was unable to find by electrophoresis a comparable protein in the bleb fluid or plasma of the two patients of this series examined by her. In examining the bleb fluid of nine of the burned and one frostbitten patient, Doctor Zamecnik found 11-fold differences in peptidase activity indicating the occasional release of such intracellular enzymes in the wounds of human beings as well as in dogs. Our search for other abnormal proteins in burned patients by measurement of cholinesterase and amylase activity of bleb fluid and plasma proved negative.

CONCLUSIONS AND SUMMARY

The nature of the shift of protein from the vascular bed into the burn wound has been examined experimentally and in the burned patient. In the dog the flow of protein through the wound has been measured by collecting lymph and in the patient by comparison of plasma and bleb fluid. Comparable results with the same implications have been found in the dog and the human being.

Proteins and fluid are in continuous slow circulation through the burn wound, returning to the blood stream via the lymphatics. The free flow in the lymphatics apparently accounts for the rapid resorption of edema in the uninfected burn wound after 48 hours;[23] as the capillary membrane heals, resorption is more rapid than the filtration from capillary into wound.

The total protein concentration of fluid in the burn wound is always lower than that of the plasma; more water than protein is lost from capillary into wound. The initial change in the residual circulating plasma is therefore an increase in concentration of total protein. This more concentrated plasma presumably exerts an increased colloid osmotic pressure in the unburned regions of the body. Following resorption of water from unburned tissues and fluid therapy, this increased concentration of the proteins of the residual plasma is replaced by a dilution.

The initial increase in protein concentration and osmotic power of the circulating plasma is not encountered following a hemorrhage of whole blood where the entire plasma is lost.[24] For a given loss of water from the vascular bed, that is for an equal reduction in blood volume, the undamaged tissues of the burned patient face a more rapid dehydration than those of the patient following a hemorrhage.

In addition to differences in total protein concentration, there are also differences in the albumin-globulin ratio of wound fluid and plasma. These differences depend upon differential capillary permeability; a greater than normal proportion of albumin passes the damaged membrane. The wound fluid thus has a higher ratio than plasma and the plasma ratio falls initially. This fall in ratio of the plasma presumably tempers the rise in osmotic power indicated by the increase in total protein concentration.

In a search for abnormal protein as a result of the injury, enzyme systems have been investigated in both plasma and lymph. An increase in activity was found of a peptidase of intracellular type in dog lymph following burning with divergent activities in bleb fluid of the human being.[13] The amylase and cholinesterase systems of blood and lymph of the dog were also studied before and after burning. Neither evidence of disturbance of these enzyme systems nor of preferential passage of these proteins through the damaged capillary membrane was found.

REFERENCES

[1] Drinker, C. K., and J. M. Yoffey: Lymphatics, Lymph and Lymphoid Tissue. Harvard University Press, Cambridge, 1941.

[2] Field, M. E., C. K. Drinker and J. C. White: Lymph Pressures in Sterile Inflammation. J. Exper. Med., **56**: 363, 1932.

[3] Glenn, W. W. L., D. K. Peterson and C. K. Drinker: The Flow of Lymph from Burned Tissue, with Particular Reference to the Effects of Fibrin Formation upon Lymph Drainage and Composition. Surgery, **12**: 685, 1942.

[4] Cope, O., and F. D. Moore: A Study of Capillary Permeability in Experimental Burns and Burn Shock Using Radioactive Dyes in Blood and Lymph. J. Clin. Investigation, **23**: 241, 1944.

[5] McMaster, P. D., and S. S. Hudack: The Participation of Skin Lymphatics in Repair of the Lesions Due to Incisions and Burns. J. Exper. Med., **60**: 479, 1934.

[6] McMaster, P. D.: Lymphatic Participation in Cutaneous Phenomena. Harvey Lectures Series, **37**: 227, 1941–42.

[7] Cope, O., J. B. Graham, G. Mixter, Jr., and M. R. Ball: An Experimental Study of the Threshold of Thermal Trauma and of the Influence of Adrenal Cortical and Posterior Pituitary Extracts on the Capillary and Chemical Changes. To be published.

[8] Rhinelander, F. W., J. L. Langohr and O. Cope: Explorations into the Physiologic Basis for the Therapeutic Use of Restrictive Bandages in Thermal Trauma. To be published.

[9] Perlmann, G. E., W. W. L. Glenn and D. Kaufman: Changes in the Electrophoretic Pattern in Lymph and Serum in Experimental Burns. J. Clin. Investigation, **22**: 627, 1943.

[10] Rawson, R. A.: The Binding of T-1824 and Structurally Related Diazo Dyes by the Plasma Proteins. Am. J. Physiol., **138**: 708, 1943.

[11] Tobin, L. H., and F. D. Moore: Studies with Radioactive Di-Azo Dyes. II. The Synthesis and Properties of Radioactive Di-Brom Trypan Blue and Radioactive Di-Brom Evans Blue. J. Clin. Investigation, **22**: 155, 1943.

[12] Howe, P. E.: The Use of Sodium Sulfate as the Globulin Precipitant in the Determination of Proteins in Blood. J. Biol. Chem., **49**: 93, 1921.
Idem.: The Determination of Proteins in Blood. A Micro-Method. *Ibid.:* **49**: 109, 1921.

[13] Zamecnik, P. C., M. L. Stephenson, and O. Cope: Peptidase Activity of Lymph and Serum After Burns. J. Biol. Chem., **158**: 135, 1945.

[14] Cullen, G. E., and D. D. Van Slyke: Determination of Fibrin, Globulin and Albumin Nitrogen of Blood Plasma. J. Biol. Chem., **41**: 587, 1920.

[15] Harkins, H. N.: The Treatment of Burns. Charles C. Thomas, Springfield, Ill., 1942.

[16] McIver, M. A.: A Study in Extensive Cutaneous Burns. Annals of Surgery, **97**: 670, 1933.

[17] Cope, O.: Symposium on the Management of the Cocoanut Grove Burns at the Massachusetts General Hospital. The Treatment of the Surface Burns. Annals of Surgery, **117**: 885, 1943.

[18] Cope, O.: The Chemical Aspects of Burn Treatment. J. A. M. A., **125**: 536, 1944.

[19] Phillips, R. A., D. D. Van Slyke, V. P. Dole, K. Emerson, Jr., P. P. Hamilton and R. M. Archibald: The Copper Sulfate Method for Measuring Specific Gravities of Whole Blood and Plasma. With Line Charts for Calculations of Plasma Proteins, Hemoglobin and Hematocrit from Plasma and Whole Blood Gravities. Bumed News Letter of the U. S. Navy, 1943.

20 White, A., and T. F. Dougherty: The Pituitary Adrenotropic Hormone Control of the Rate of Release of Serum Globulins from Lymphoid Tissue. Endocrinology, **36:** 207, 1945.
21 Selye, H.: Studies on Adaptation. Endocrinology, **21:** 169, 1937.
22 To be published.
23 Cope, O., and F. D. Moore: The Redistribution of Body Water and the Fluid Therapy of the Burned Patient. ANNALS OF SURGERY, **126:** 1010, 1947.
24 Elman, R., and H. Riedel: Hemodilution Following Experimental Hemorrhage. Influence of Body Movement, of the Ingestion of Water and of Anesthesia Induced by Intravenous Administration of Pentothal Sodium. Arch. Surg., **53:** 635, 1946.