THE INFLUENCE OF THE PLASMA COLLOIDS ON THE GRADIENT OF CAPILLARY PERMEABILITY

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The wall of the capillary has long been considered on good evidence as behaving like an indifferent semipermeable membrane separating the blood from the tissues. All along the course of the little vessel the permeability has been supposedly the same, differences in the happenings at various points being referable to the interplay of several factors, diffusion, hydrostatic pressure, and osmosis chief among them. It has been held that in the first portion of the capillary the force of hydrostatic pressure dominates over the osmotic pressure of the blood colloids, with the result that fluid is forced out into the tissue to be reabsorbed again as the vein is approached, because the drawing power of the colloids there prevails. The recognition that the permeability of the capillaries of some organs—muscle, skin—increases progressively in the direction of flow (1) has necessitated a reconsideration of the rôle of the factors just referred to. Recent experiments have shown that the gradient of capillary permeability exists independently of the hydrostatic pressure (2). The present work was undertaken to disclose the relation to it of osmotic conditions and especially of the influence of the osmotic pressure of the blood colloids.

Methods

The effect on the gradient of capillary permeability of greatly modifying the percentage of the circulating blood proteins has been directly followed. For this purpose several methods were employed.

Adult rabbits of about 2000 gm., fasted 24 hours but with access to water, were used throughout. They were anesthetized with sodium iso-amyl-ethyl barbiturate (sodium amytal—Eli Lilly and Co.) given intravenously, 0.5 to 0.7 cc. of 10 per cent solution per kilo. Freshly prepared solutions were always employed. For

surgical procedures light ether was required in addition. Loss of heat was prevented by keeping the animal on a warm pad.

Chicago blue 6B was the dye of choice, and voluntary muscle the tissue observed. As proven in a previous paper (1) specific affinities are not responsible for the staining, for the dye passes directly from the blood to the interstitial fluid, the initial coloration of the muscle being due solely to its presence in this fluid. It is poorly diffusible and the gradient of its escape is plainly visible in the gross color pattern. The initial escape of the dye, which is restricted to the further portion of the capillaries, can be followed with the microscope.

The warmed isotonic solution of purified Chicago blue (7 per cent in 0.45 per cent NaCl) was introduced in approximately 3 minutes; 7 minutes later the carotids were cut and the muscles examined at once. The general technique of examination has already been described, as also the color pattern indicative of the gradient of permeability along the muscle capillaries (1),—regularly distributed blue bars in long muscles, and a fern-like pattern in the sheet muscles of the abdomen, attesting to the progressive increase in the escape of dye along the further part of the capillaries. Variations in the rate and abundance of escape are frequent in normal animals; and hence only marked and constant departures from the characteristic findings can be deemed significant.

Some of the experimental procedures entailed important changes in the blood volume. The cell-plasma ratio furnished a rough index to them. To obtain this ratio duplicate blood specimens taken from the ear into standard 0.04 cc. pipettes were diluted with 2 cc. of 0.9 per cent NaCl in Hamburger tubes (3) and the proportion of cells determined after centrifugation. By this method changes in the cell volume due to alterations in the tonicity of the serum were avoided. In all calculations the normal blood volume of the rabbit was assumed to be 5.5 per cent of the body weight (4).

The carotid blood pressure was followed with a kymograph. Blood proteins were determined by the method of Van Slyke, using specimens taken from an ear vein. In certain cases the percentages of both albumin and globulin were determined in order to apply the osmotic pressure formula of Govaerts (5).

Effects of Increasing the Plasma Proteins

The recent work of Adolph and Lepore (6) has shown that during the readjustment of blood bulk after introduction of hypo- and hypertonic solutions into the circulation, the voluntary muscles gain and lose fluid respectively. It is well known that the plasma constituents of transfused blood begin to leave the circulation almost at once, and it seems not improbable that some portion enters the muscle. If this is the case, the conditions affecting distribution of dye to this tissue during the readjustment after transfusion may be very different from those obtaining when the blood bulk has become stationary. For

this reason we have in some instances injected dye after the readjustment had been accomplished and in others while it was still in progress.

By the intravenous injection of large amounts of compatible heparinized plasma, or in some instances of serum, it proved possible to increase the concentration of the plasma proteins by as much as 40 per cent. This came about through a greater intravascular retention of protein than of the other plasma constituents. The procedure involved a considerable though transitory increase in blood volume. With the aim of avoiding this we worked out a method whereby the proteins of the blood could be concentrated *in vitro* for injection.

Concentration of the Blood Proteins

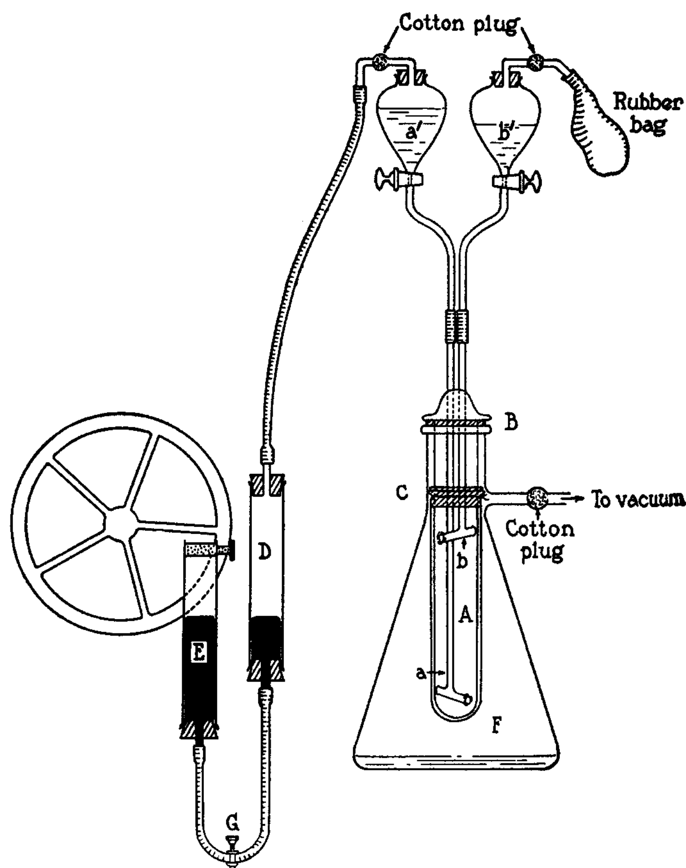
The fresh sterile sera of a number of rabbits are pooled and introduced into a specially devised concentrator.

A is a broad glass tube about 4 cm. in diameter and 18 cm. long, with one end rounded and the other expanded into a cap which rests at its edge on a ring of rubber (*B*), thus closing the filter flask (*F*) into which the tube is introduced. Two small tubes, *a* and *b*, pass down through the large one to end in cross-tubes which, extending through its wall, open near the top and bottom respectively. They terminate above in the bulbs *a′* and *b′*, the serum reservoirs. All the connections with *A* are sealed in.

A collodion tube slightly larger than the glass one, made according to Brown's (7) method, and just retaining proteins while letting crystalloids through freely ("80 per cent membrane"), is treated with 80 per cent alcohol and tied over *A* upon a rubber ring *C*. A close-fitting gauze jacket affords it support, preventing rupture when the pressure is lowered in the filter flask. This jacket does not appear in the diagram. The gauze has been previously sterilized and shrunken by boiling. About 2 mm. clearance should exist between the glass tube and the collodion surface, which latter has a filtering area of 250 sq. cm.

The apparatus is washed through by way of the serum reservoirs, first with 60 per cent alcohol, then with double distilled H_2O, and lastly with sterile 0.9 per cent NaCl solution. With the stop-cocks open, serum is now poured into one of the reservoirs, filling the space between the collodion tube and the glass tube *A* and mounting into the other reservoir. When both have been half-filled in this way, *a′* is connected with a pair of short vertical cylinders, *E* and *D*, containing mercury and joined by rubber tubing. The glass connection leading away from *a′* is sterile and is provided with a bulb packed with cotton to filter the air. Vertical excursion of the mercury columns in the cylinder *D* is provided for by attaching *E* to the circumference of a 10-inch wheel making 2 revolutions per minute, and the amount of the excursion is controlled by a screw-clamp, *G*. By this means pressure is brought to bear alternately on the serum in the reservoir *a′*, forcing it down into the space

between glass and collodion or sucking it up again. The excess displacement is taken care of by the reservoir b' which is connected with a sterile rubber bag to prevent free entry of the atmospheric air while permitting change of the serum level. The result is that the fluid in contact with the collodion surface is constantly changed. If now the pressure in the filter flask F is diminished to 400 mm.



TEXT-FIG. 1

Hg by means of the laboratory vacuum, filtration proceeds rapidly,—at a rate of about 4 cc. per minute to begin with, and somewhat more slowly as concentration progresses. The entire operation is carried out in an ice box at 2.5°C. In this way several hundred cubic centimeters of serum can be concentrated to about a fifth of its bulk within a few hours. The ultrafiltrate is sterile, colorless, protein-free and has the same freezing point as the original serum. The concentrate is

brownish red,—the ruddy tint being referable, as the spectroscope shows, to hemolysis imperceptible in the original serum,—but clear and not at all syrupy. The close agreement of the freezing points of ultrafiltrate and original serum, as well as the bulk of the separated fractions, attests the absence of any significant evaporation. This could be eliminated entirely by introducing into the flask a layer of sterile, washed paraffin oil, through which the ultrafiltrate would sink.

For determination of the protein concentrations in plasma and concentrate, Van Slyke's method was employed as has already been mentioned. Amounts of protein up to twice that contained in the animal's own total plasma were injected at body temperature into an ear vein of the rabbit used in the experiments. A test was made beforehand to find whether the concentrate hemolyzed or agglutinated the prospective recipient's blood. Hemolysis was never met and agglutination but rarely. In the latter case a compatible recipient was chosen. Since the serum had been outside the body more than 24 hours during the concentration there was no reason to expect the untoward effects recorded by Freund (8) for fresh serum; nor were they encountered. The injection was always well tolerated and the blood pressure remained high.

Hematocrit specimens taken after the injection of concentrate showed a diminution in the percentage of red cells much more considerable than could be accounted for by the fluid bulk introduced. It was plain that the increase in circulating protein had caused fluid to enter the blood from the tissues. The dye injection was not made until the blood volume had become approximately stationary. Hematocrit readings at this time indicated that the increase in volume was not great enough to reduce the protein percentage to that existing prior to the injection, and blood specimens taken just prior to dye injection proved that the concentration of protein per cc. of blood was indeed much increased, by as much as 40 per cent in individual instances, as already stated.

SPECIMEN PROTOCOLS

Rabbit Injected with Normal Plasma.—A male rabbit weighing 1200 gm. was fasted for 24 hours but allowed water. At 2:15 p.m. 0.7 cc. of 10 per cent amytal was given intravenously. Prompt hypnosis occurred, with a somewhat depressed respiratory rate. At 2:35 a first blood specimen was taken from the ear and at 2:50 the carotid was cannulated and the blood pressure tracing started. At 3:15, with the blood pressure at 70 mm. of Hg the injection of warmed heparinized plasma was begun. The first 20 cc. was given rapidly (in 2½ minutes) and the pulse rate slowed considerably, while the blood pressure rose. The injection was continued at a slower rate until 62 cc. in all had been introduced in 10

minutes. The blood pressure was now 80 mm. Hg. The animal was covered to conserve warmth and blood specimens were taken at intervals. The table shows the changes.

Time	Red cells	Serum protein	Blood pressure
p.m.	*per cent*	*gm. per cent*	*mm. Hg*
2:35	35	5.58	70
3:15	(62 cc. of plasma injected between 3:15 and 3:27)		
3:30	21	7.12	80
3:45	25		75
4:00	26		
4:30	27		73
5:00	27		
5:30	27		
6:00	27		65
6:30	26		Clotting in cannula
7:00	26		
7:30	26	7.58	

Rabbit Injected with Serum Concentrate.—A female rabbit weighing 1520 gm. was fasted for 24 hours. The red cell percentage was 43 and the serum protein 6.3 gm. per cent. At 1:40 p.m. 0.5 cc. of 10 per cent amytal was given intravenously. At 2:08 p.m. the carotid was cannulated; and at 2:21 the injection of warmed concentrated serum (24.7 gm. per cent protein) was begun. 12 cc. was injected in 4 minutes. An hematocrit reading taken 6 minutes later showed a red cell percentage of 34. At 2:50 the reading was 33, and at 3:00 again 34. The serum protein was now 8.4 gm. per cent.

In the animals of the foregoing protocols and in others treated in the same way, no dye was given. They were utilized merely to determine the course of the readjustment.

In the succeeding experiments dye was given at a time when the successive hematocrit readings indicated that the blood volume had become stationary. The amount of Chicago blue put into circulation was the same per cc. of plasma that yielded in normal rabbits a well marked coloration having the pattern indicative of the gradient of vascular permeability. Since staining intensity is conditional upon the concentration of dye in the plasma, allowance had to be made for the increase in plasma volume. This was calculated from the change in the red cell percentage, assuming that the original blood volume amounted to 5.5 per cent of the body weight. We were interested, not

in the eventual distribution of dye throughout the tissue of the rabbit, but only its first escape from the blood. Consequently the fact that the amount of stainable tissue was unaltered by the procedure could be disregarded.

The staining encountered in animals treated as described and killed after the routine period of 10 minutes from the beginning of the injection showed significant and constant deviations from the normal. The coloration was less intense than in normal rabbits and more narrowly localized to the furthest capillary region—the region of greatest permeability. One may liken the picture to that of the staining in a normal rabbit with one wash of color removed.

In the experiments just described conditions had become stabilized, the readjustment of the blood bulk having ceased. In the following experiments dye was given together with the concentrate and its escape occurred while the readjustment was going on. The change in the percentage of circulating protein could not be determined directly in these cases because the blood carried a nitrogen-containing dye.

A male rabbit weighing 1860 gm. was given amytal at 2:15 p.m. By 3:01 p.m. the carotid artery had been cannulated and connected with the manometer. At 3:10, 4.5 cc. of an isotonic watery solution of Chicago blue and 2.5 cc. of concentrate were injected in 4 minutes and 40 seconds. After another 20 seconds a further injection of concentrate alone was begun and 8 cc. more of it was introduced in 8 minutes and 30 seconds. The skin vessels showed moderate dilatation as the injection proceeded. The carotids were cut 2 minutes after it ended. Autopsy showed the familiar, barred, blue pattern in the voluntary muscles with the bands somewhat narrower and of a lighter hue than under ordinary circumstances.

As control a 1900 gm. rabbit was anesthetized lightly with ether and given 3.7 cc. of the same dye solution in 3 minutes. The carotids were cut after exactly the same lapse of time as in the experiment just described. Autopsy showed the usual, relatively pronounced staining.

In Table I the blood changes in the animals of the two groups of experiments are summarized.

Effects of Hypertonic Solutions

To supplement the observations on the effects of increasing the plasma proteins, several experiments were made upon the course of the staining as influenced by the injection of a hypertonic solution of glu-

TABLE I

The Blood Changes in Animals with Increased Plasma Proteins

Rabbit		Body weight	Plasma volume	Amount of plasma or concentrate injected	Initial red cell percentage	Expected red cell percentage after injection	Actual red cell percentage stabilized	Elapsed period		Expected change in blood volume	Actual change in blood volume	Initial plasma protein concentration	Initial plasma protein	Amount of protein injected	Expected plasma protein concentration	Actual plasma protein concentration after injection	Elapsed period		Percentage change in protein concentration
A. Animals in which the blood bulk had become readjusted before dye was given																			
		gm.	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*hrs.*	*min.*	*per cent*	*per cent*	*gm. per cent*	*gm.*	*gm.*	*gm. per cent*	*gm. per cent*	*hrs.*	*min.*	*per cent*
Injected with plasma	P_1	1300	39	65	45	*23*	*33*	4	5	*+96*	*+36*	7.7	3.0			*7.9*	4	5	+3
	P_2	1200	42	62	36	*19*	*26*	4	5	*+89*	*+38*	5.58	2.3			*7.58*	4	5	+36
	P_3	1440	51	72	36	19				*+89*		6.73	3.4	4.7	*6.6*	*7.74*	4	50	+15
	P_4	1270	47	64	33	*17*	*33*	4		*+94*	*0*	*6.6*	3.1	4.2	*6.6*	*7.2*	4	55	+9
Injected with serum concentrate	B	1520	48	12	43	**38**	**34**		35	**+13**	**+26**	6.25	3.0	3.0	**10.0**	**8.38**		30	+34
	C	1740	59	15	38	**33**	**28**		30	**+15**	**+36**	6.14	3.6	4.2	**10.5**	**7.28**		30	+19
B. Animals in which readjustment was taking place																			
	A	1860	64	11	37	**33**	**29**		3	**+12**	**+28**			2.8					
	D	1600	53	25	40	**31**	**28**		2	**+29**	**+43**			6.1					
	E	1680	52	14	44	**38**	**34**		3	**+16**	**+29**			3.5					

cose. The injection was intermitted while the dye was placed in circulation but was then resumed and continued until sacrifice of the animal. The fact is well attested that the readjustment of the fluid relationships is exceedingly rapid after the introduction of non-isotonic solutions of crystalloids. In order to ensure a continuous passage of fluid from the tissues to the blood while the dye was distributing itself, the glucose solution was continuously but slowly injected during the entire period from immediately after the dye injection until the animal was killed.

Specimen Protocol.—A rabbit weighing 1675 gm. was fasted for 24 hours. At 2:25 p.m. the red cell percentage was 44. At 2:32, 1 cc. of 10 per cent amytal was given intravenously; and at 3:15 the carotid was cannulated. The blood pressure was 90 mm. of Hg. At 3:32, 2 cc. of 70 per cent glucose was given in 60 seconds into an ear vein. At once thereafter injection of the calculated dose of Chicago blue was begun, and when this was completed, after 2½ minutes, the glucose injection was resumed and continued for 7½ minutes. By that time 11 cc. of 70 per cent glucose had been injected in all. The animal was now exsanguinated by cutting the carotids. The red cell percentage as obtained on the carotid blood was now 36, indicating a 22 per cent increase in the blood volume and a 40 per cent increase in the plasma volume. At autopsy the muscles showed the pattern characteristic of the gradient of vascular permeability, the findings not differing noticeably from the normal. The skin was deeply colored and there was no observable dryness of the tissues. The bladder was full and there was a small amount of dye in the urine.

Despite the great increase in the plasma volume of this animal, an increase which involved, of course, a passage of fluid from the tissues to the blood, as well as a dilution of the dye in the blood, the staining of the muscles and skin did not differ from the normal either in intensity or in distribution. The skin is known to supply water to the blood in quantity when hypertonic solutions are given, and Adolph and Lepore's recent work indicates that the muscles yield some of it. The reason for the difference in the findings when the blood volume has been increased with plasma and with glucose respectively is dealt with further on. For the present it will suffice to point out that the movement of fluid from the tissues to the blood does not essentially alter the gradient of vascular permeability or check the process of staining. The findings yield proof in addition to that of previous work (2) that the spread of dye from the blood to the tissues cannot be essentially dependent upon fluid movement, as some have supposed.

Effects of Reducing the Plasma Proteins

The percentage of circulating plasma proteins can be readily reduced by plasmapheresis. As ordinarily carried out this involves the repeated return of washed red cells suspended in Locke's solution. There is much in the literature to suggest that the best of salt solutions may harm the capillary endothelium, and it is essential to prevent such injury if one is to measure capillary permeability. In an initial series of experiments recourse was had to the ability of the animal to make up its own blood bulk after each removal of cells. Robertson and Bock have shown that forcing water by the alimentary tract after hemorrhage results in a remarkably rapid and effective restoration of the blood volume (9). Utilization was made of this phenomenon.

Warmed water or Ringer's solution was administered to amytalized rabbits by stomach tube, to the amount of 120 cc., and soon afterwards the anesthetized animal was bled repeatedly at short intervals and the cell sediment obtained on centrifugation was returned to the body by way of a femoral vein. To replace the loss of the first bleeding the cells from a compatible donor were used. Thereafter those of the host were injected, as obtained at each previous bleeding. The bleedings were carried out rapidly from a carotid artery into a 1 per cent heparin solution (1 cc. to 30 cc. of blood). The mixture was centrifuged and the plasma pipetted off as completely as possible. The cells were then suspended in 0.9 per cent NaCl, recentrifuged, the fluid discarded, and the sediment filtered through gauze before injection to remove the small amount of white granular fibrin which was always present in spite of the heparin. It was found that by the repeated bleedings and reinjections the plasma proteins could be reduced by about one-third in the course of 3 hours. The blood pressure tended to fall gradually but there was no other evidence of circulatory disturbance. The blood volume underwent a slight decrease as indicated by changes in the proportion of red cells in hematocrit specimens.

The amount of dye to be introduced was calculated on the basis of the plasma volume existing at the time of the injection. The blood pressure tracing was continued until the animal was killed.

In some further experiments the washed cells were made up to the original volume by the addition of ultrafiltrate obtained as a by-product to the concentration of protein by the method already described. Although the plasma was thus replaced by a fluid which lacked only the proteins of the blood, the blood pressure and volume fell to approximately the same extent as when no addition had been made to the cells prior to their reinjection.

Incidentally to the work, a number of tests were made upon rabbits of the merits of ultrafiltrate and of Ringer-Locke solution respectively, in restoring blood volume after hemorrhage. The carotid tracings did not indicate that the ultrafiltrate had any special advantages.

Chicago blue 6B, given when the blood protein had been much reduced by plasmapheresis and the blood volume had become stationary, yielded a staining of the muscles notably greater than normal. This

TABLE II

The Blood Changes in Animals with Decreased Plasma Proteins

Rabbit	Body weight	Plasma volume	Amount of plasma removed	Elapsed time		Initial red cell percentage	Final red cell percentage	Elapsed time after last removal of plasma		Percentage change in blood volume	Initial plasma protein concentration	Initial plasma protein	Approximate amount of protein removed	Final plasma protein concentration	Elapsed time after last removal of plasma		Percentage change in protein concentration
	gm.	*cc.*	*cc.*	*hrs.,*	*min.*	*per cent*	*per cent*	*hrs.,*	*min.*	*per cent*	*gm. per cent*	*gm.*	*gm.*	*gm. per cent*	*hrs.,*	*min.*	*per cent*
L	1750	62	67	2	30	36	43		17	−16	6.36	4.0	3.4	3.79		17	−42
M	1920	65	97	3		38					6.93	4.5	5.0	3.37		13	−51
N	1500	43	42	1	45	48	39		30	+23	7.48	3.2	2.9	6.4		30	−14
O	1900	65	60	2	37	38	39	1	20	−2	6.24	2.7	2.9	3.35	1	20	−46
P	1830	58	56	2	42	42	43	1	42	−2	5.65	3.3	2.6	3.63	1	42	−36
Q	1830	62	54	3		38	39	2	55	−2	6.11	3.8	2.8	3.58	2	55	−41
R	1850	61	60	3		40	43	2	45	−7	5.82	3.4	2.9	3.76	2	45	−35
X	2300	80	75	2		37	40		20	−7	6.13	4.8	3.5	3.16		20	−48
T	2000	74	74	3		39	40	1	10	−2	5.8	3.9	3.3	3.18	1	10	−45
U	1850	61	105	3	24	40	46		10	−13	5.82	3.6	4.4	2.63		10	−55
V	1980	64	78	1	45	41	43		2	−5	5.8	3.7					
W	1900	67	74	1	40	36	48	1	10	−25	6.13		3.0	4.04	3		−34

happened although the systemic blood pressure had fallen somewhat as a result of the lessening in blood bulk. The bars indicative of the gradient of permeability were broader and more intense than ordinary, and in addition to them there was some generalized staining. In summary of the findings, one can say that the staining was like that in a vitally stained normal rabbit but with an extra wash of color everywhere superimposed.

Table II presents the blood findings in the animals treated as just described.

The Influence of Adsorption of the Dyes

The experiments prove that Chicago blue escapes more readily from the blood when its plasma proteins have been reduced, and with greater difficulty when they have been increased. One cannot conclude forthwith, however, that the differences are the result of a lessened or increased colloid osmotic pressure; for the possibility exists that they are due, in part at least, to adsorption of the dye on the plasma proteins. Marshall and Vickers have found that ultrafiltration of an aqueous solution of phenolsulfonphthalein through a collodion membrane yields a fluid of the same dye concentration as the original solution, but that ultrafiltration of a plasma mixture yields a more dilute filtrate (10). They suggest that certain phenomena in the renal elimination of phenolsulfonphthalein may be due to adsorption of the dye on the plasma proteins. Grollman showed that phenol red is indeed adsorbed on the blood proteins, more especially on the albumin fraction (11).

Tests for the fixation of Chicago blue on blood proteins by the method of ultrafiltration through collodion membranes are rendered difficult by the indiffusibility of the dye. Even through highly permeable membranes little passes from an aqueous solution in the course of several days. However, the diffusion method of Northrop and Anson in which a partition of porous glass is utilized (12), proved admirable for our purpose.

The cell, sealed with the porous glass, was filled with fluid containing a known amount of dye, and the stop-cock was closed, thus preventing alterations in the volume of the fluid by osmotic transport of water, which might have interfered with the free diffusion of the dye. An ultrafiltrate of rabbit serum, prepared in the way already described, was run into the outer jacket in sufficient quantity just to cover the porous disc. The fluids had been cooled previously to 2.5°C. and the apparatus was kept at this temperature.

The ultrafiltrate outside of the cell was drawn off and replaced at regular intervals of time and the amount of dye that had passed into it was estimated colorimetrically. This amount became constant when the concentration gradient of dye in the membrane had established itself, and this in turn was a function of the diffusibility of the dye and of the concentration within the cell of its freely diffusible portion, as distinct from that adsorbed on the blood proteins. The porous disc employed was 0.5 mm. thick and impermeable to proteins within the time limit of our experiments, as proven by preliminary tests specifically directed to the point.

Equimolecular solutions of Chicago blue in ultrafiltrate and in rabbit serum were employed successively within the cell, and possible changes in the permeability of the membrane resulting from adsorption upon it of protein were controlled by a second series of determinations with dye in ultrafiltrate, carried out immediately after the cell had been emptied of serum, without washing it. In all instances the dye was present in the amount per cc. that had been introduced into the plasma of the rabbits used in our experiments.

The colorimetric determinations showed that the dye diffused more than three times as slowly from serum as from ultrafiltrate. Consequently the specimens of diffusate were removed every hour in the case of serum, and every 15 minutes in the case of the ultrafiltrate. Each specimen, as obtained, was made up to 25 cc. with Ringer's solution and compared in a Duboscq colorimeter with a 1:16,000 dilution of Chicago blue in Ringer's solution. During the readings the column of diffusate was kept constant (at 35) and that of the standard was varied. It follows that the figures procured are directly indicative of the relative concentration of dye in the various specimens. Thus for example the concentration of dye in a first specimen of ultrafiltrate, as recorded in the type instance of Table III, was nearly twice as great as in the last of the same series (25 as compared with 14).

TABLE III

Influence of Plasma Proteins on the Diffusion of Chicago Blue 6B

	Dye in ultrafiltrate (15 min. specimens)	Dye in serum (hr. specimens)	Dye in ultrafiltrate (15 min. specimens)
Period of adjustment	25	41	37
	21		25
	18		21
	16		19.5
	15	32	16
			15
		25	
		21	
Period of diffusion at constant rate	14	19	14
	14		14
	14		14
	14	19	
		19	
Relative amounts of dye escaping per hr. during constant period	**56**	**19**	**56**

It will be seen from Table III that dye diffused with far greater rapidity from the filtrate than from the serum. Since the amount of dye passing in unit time through the disc after the concentration gradient becomes uniform can be taken as a measure of the freely diffusible dye, it would appear that, in the instance given, as in others not here recorded, only about 30 per cent of the Chicago blue in the serum mixtures was available for diffusion, the remainder being adsorbed on the plasma proteins. The objection that the pores of the disc had become clogged with protein particles is disposed of by the control observations made after the use of a serum-dye mixture. The figures indicate that there was no mechanical clogging; indeed more fluid came through into the first control specimen than into the corresponding one of the preliminary series.

By a test of another sort the adsorption of Chicago blue was readily demonstrated. A marked reduction of the tinctorial value of phenol red is associated with its adsorption upon the blood colloids. The same holds true of Chicago blue, dilute solutions in serum being less intensely colored than similar ones in Ringer's fluid. To test whether this holds true when the dye is present in the amount per cc. of plasma employed in our animals, it has been necessary to utilize thin layers of fluid, since the deep color of the solutions tends to mask differences between them. Small drops of serum and of salt solution containing dye in the same appropriate quantity were placed side by side in a hemocytometer chamber having a double dais. Comparison showed a pronounced difference in their intensity of color, the mixture with salt solution being by far the deeper blue. No attempt was made to quantitate the difference.

DISCUSSION

The experiments show that when the percentage of the blood proteins is experimentally increased, the passage of Chicago blue from the muscle capillaries into the tissue takes place more slowly than usual and is confined in the beginning to the furthest portion of these vessels. When the proteins are decreased the dye escape is more abundant than usual and extends further back along the capillaries. In both cases the staining pattern is still indicative of an increasing permeability of the vessel wall as the venule is neared.

Are the altered osmotic conditions resulting from increase or decrease of the blood colloids responsible for these changes? Assuming this one might suppose that the passage of water from the tissue to the blood, consequent upon a sudden experimental increase in the blood proteins, would involve a flow in the opposite direction to that of dye escape, hindering the latter. But as a matter of fact, when dye is injected together with a hypertonic solution of glucose, and water is rapidly drawn into the circulation while dye passes out into the tissues, one finds no alteration in the distribution of dye to muscle or skin. The injection of dye concurrently with a plasma concentrate rich in blood proteins yields a staining quite as good as when the circulation has become stabilized after introduction of the concentrate, although water is doubtless passing from the muscle into the blood (6) while the coloration is going on.

If the osmotic pressure of the blood proteins does not condition the escape of dye, what can be the cause for the alterations encountered when the protein percentage has been changed? Our tests indicate that the amount of blood protein upon which the dye can become adsorbed is the effectual factor. Chicago blue when thus adsorbed is largely removed from the possibility of diffusion, as shown by our *in vitro* experiments; and the rate of staining with a dye is conditional not only upon its diffusibility, but upon the available amount of it (13, 1). The intensity of the staining of muscle with Chicago blue, the effective extent of the gradient of capillary permeability, and the presence or absence of diffuse coloration along the capillaries in addition to the local staining referable to the gradient, all have been found to vary with the amount of dye in circulation. The effective amount, in our experiments involving alterations in the per cent of plasma proteins, varied with this percentage, more or less being available accordingly as the percentage of circulating blood protein was decreased or increased.

It might be urged that adsorption upon tissues must also be reckoned with as complicating the findings; but Chicago blue is not immediately adsorbed in perceptible amounts upon the tissue of muscles into which it escapes (2). One has then only to consider adsorption upon the blood proteins, and this will account for the observed differences in the amount of dye escaping from the blood. The most striking evidence of

the influence of adsorption upon the proteins was obtained in the plasmapheresis experiments in which no fluid was added to the red cells restored to the circulation. In these experiments the carotid blood pressure fell, owing in part at least to a gradual lessening of the blood bulk. From previous knowledge it seems certain that there had been some compensatory restriction of the muscle circulation (14). Nevertheless the escape of dye from the capillaries was more abundant and the extent of the gradient of permeability was greater than under normal conditions, while, furthermore, dye escaped everywhere along the capillaries as does not happen ordinarily,—all this being due, it would appear, to the diminution in blood protein, which left more of the injected dye available for diffusion.

Can it be that the greater and greater escape of dye as the blood carries it along the capillary way, an increase essentially independent of the hydrostatic pressure (2) and of osmotic conditions, is caused by a progressive falling off in the amount of dye adsorbed upon the blood proteins as the blood flows along, with result that more becomes available progressively for escape through the capillary wall? Such a view entails the assumption that the changes taking place in the blood in its passage from arteriole to venule are such as would act to lessen adsorption. If this were the case, the gradient should vary directly with the magnitude of these changes, being less marked and even absent in proportion as they are prevented. Several facts negate this possibility. After section of the nerves to mammalian muscle the gradient persists although the blood undergoes but little alteration during its rapid passage through the dilated capillaries. The blood entering the capillaries of frog skin is essentially venous, having the lowest tension of oxygen and the highest of carbon dioxide to be found anywhere in the circulation, and it is rendered arterial on its way to the venules. Despite this reversal of the ordinary condition of affairs along capillaries, dyes escape in greater and greater amount from these vessels as the venules are neared, just as in the case of mammalian capillaries. Furthermore, when the circulation is cut off after dye has been allowed to enter the vessels of the mouse's ear, the staining that gradually ensues from capillaries dilated as the result of a previous ischemia shows that the gradient of dye distribution still exists, although now the stagnant blood has become venous everywhere. These facts would seem to rule

out the possibility that the progressive release of adsorbed dye is responsible for the increasing escape along the capillaries.

The fact that the gradient of capillary permeability exists independently of alterations in the percentage of the blood proteins is, of course, far from meaning that these are devoid of effect upon exchange between the blood and tissues. The edema of nephrosis and that produced experimentally by plasmapheresis (15) attest to an important influence of the proteins to hold water in the circulation as do some of the phenomena of the present experiments, notably the persisting increase in blood bulk after the injection of serum concentrate.

In theoretical considerations of the influence of osmotic pressure upon exchange through the capillary wall, the conditions have too often been oversimplified. The capillary is far from being completely impermeable to protein (16) and it lets through much more albumin than it does globulin. Osmotic pressure must always be referred to the particular membrane with which it is measured, for the osmotic forces of only those elements to which the membrane is impermeable are effective. If the collodion membrane utilized in osmotic pressure determinations held back all electrolytes, instead of the protein only, serum would give a value of 7 atmospheres instead of 40 mm. of Hg. As it is, collodion membranes impermeable to all the proteins are employed in tests for the osmotic pressure of serum, not membranes which let albumin through with relative ease; and these tests are made against an ultrafiltrate devoid of protein, not one containing it as lymph actually does. In consequence figures are obtained considerably higher than those to be expected had a capillary wall been utilized. The fact that this wall is not everywhere equally permeable, but becomes progressively more so as the venule is neared, complicates the state of affairs.

It is pertinent to inquire whether adsorption upon plasma proteins may not condition the distribution to the tissues of some normal substances as it does that of our dyes. That a large proportion of the bilirubin circulating in jaundice cases is adsorbed upon the proteins has recently been recognized (17).

In previous work the fact has been brought out that the escape of vital dye in progressively increasing amount along the capillaries is a phenomenon that occurs independently of alterations in hydrostatic

pressure, though its effective extent is influenced thereby. The same has now been found to hold true as concerns alterations in the percentage of plasma proteins. One must conclude, as from other evidence, that the gradient of vascular permeability disclosed by the escape of dye cannot be due to an interplay of hydrostatic and osmotic forces such as has generally been held to control exchange between the blood and tissues. Whatever the nature of the gradient,—and the evidence points to a structural cause for it,—it is a dominating factor in exchange.

SUMMARY

The influence of osmotic conditions on the gradient of capillary permeability disclosed by the distribution into mammalian muscle of vital dyes has been tested experimentally. The percentage of circulating blood proteins was increased in rabbits by the injection of compatible plasma, or of compatible serum concentrate obtained by means of a new method of ultrafiltration which has proved both rapid and effective. It was found that when this had been done and the circulatory conditions had stabilized themselves, the gradient of capillary permeability still existed, though its effective extent was less than under normal circumstances. When the percentage of circulating blood proteins was reduced, on the other hand, by repeated bleedings with return of the cells, either as such or suspended in the protein-free fluid obtained by dialysis of serum, the extent of the gradient of capillary permeability was broadened and dye passed out into the tissue more readily than usual from the capillary as a whole. In contrast to these findings injection of a very hypertonic dextrose solution during the period when dye was escaping had no perceptible effect on the gradient of capillary permeability.

The observed phenomena cannot be explained by a flow of dye-stained fluid into or out of the blood vessels. The gradient of capillary permeability exists independently of osmotic conditions, though its extent can be markedly influenced by altering the amount of circulating blood proteins. A considerable proportion of the dye used to study the gradient is adsorbed upon these proteins, as subsidiary experiments have shown. This happening provides a sufficient cause for the differences observed in the extent of the gradient when the percentage of proteins is increased or diminished.

The evidence like that of previous papers indicates that the cause for the gradient is to be found in a structural differentiation along the capillary, such that the barrier offered by its wall progressively diminishes on the way to the venule. Most current estimates of the effective osmotic pressure of the blood proteins fail to take into account the existence of local differences in permeability along the capillary.

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