THE FLOW AND COMPOSITION OF LYMPH IN RELATION TO THE FORMATION OF EDEMA

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Present day theories of the pathogenesis of edema assign great importance to the blood proteins in maintaining normal fluid distribution between blood and tissue spaces. The original observations of Starling (1) have been confirmed and supported by the work of Epstein (2), Krogh (3), Schade and Claussen (4), Peters and his associates (5), Leiter (6), Barker and Kirk (7), and a host of others. A wealth of evidence exists to support the belief that the plasma proteins, because of the colloidal size of their molecules, are unable to diffuse to any great extent across the capillary wall and, therefore, exert an osmotic pressure which serves to prevent excess accumulation of fluid in the tissues. Evidence for the impermeability of the capillary to protein has been necessarily indirect as tissue fluid normally exists in such small amounts that it cannot be obtained for analysis. It has long been known, however, that lymph from various regions does contain protein. Within the last few years, Drinker and Field (8) have shown repeatedly that lymph from vessels which drain the subcutaneous tissues is no exception in this respect and have given reasons for their belief that this lymph possesses "an approximate degree of identity" with tissue fluid in the corresponding region. From the quantitative standpoint, at least, proof of this contention will require modification of the Starling theory.

The experiments to be described in this paper were designed to test directly the relative compositions of lymph and one form of tissue fluid. As originally conceived the plan of study was simple. Edema was to be produced in dogs, either rapidly by plasmapheresis or slowly by protein starvation, and the composition of edema fluid compared

directly with that of lymph collected from one of the lymphatic trunks draining the edematous region. This purpose has been accomplished. In addition phenomena have been encountered which on the one hand have brought a clearer understanding of the circulation through the tissue spaces and on the other have marked out features concerning which existing knowledge is still inadequate. The findings collectively form the basis of this communication.

To avoid confusion in terms it is necessary at the outset to define briefly the sense in which several words and phrases are to be employed. The word lymph will be used only when referring to the fluid content of the lymphatic vessels and not as synonymous with tissue fluid as has been done so frequently in the past. Capillary filtration, capillary filtrate, and capillary reabsorption will be employed in the exact sense which the words imply. The terms tissue fluid, interstitial fluid, and intercellular fluid will be used interchangeably to refer to the fluid which is present in the interstitial spaces and which is outside the walls of both blood capillaries and lymph capillaries. A distinction will be made between the phrases rate of lymph flow and lymph formation or lymph production. The former will apply to the volume of lymph delivered by a given lymphatic vessel in a unit time and the latter two to the process of generating fluid which under certain circumstances can enter the lymphatics and give rise to lymph flow.

Methods

The dogs used were of mongrel breeds and varied in weight from 15 to 26 kilos. Lymph was obtained exclusively from vessels of the lower leg into which cannulas were inserted some inches above the ankle but well below the knee or elbow. Lymph so obtained has not undergone any theoretical alteration by filtration through lymph nodes and comes in large part from regions liable to be affected with subcutaneous edema. In earlier experiments the small operation necessary for locating and cannulizing a lymphatic trunk was performed under general anesthesia (either nembutal or ether); in later experiments local anesthesia with novocaine was found quite sufficient. Lymph flow was stimulated either by gentle massage of the foot and ankle combined with passive motion of the extremity or by allowing the animal to walk or run about in the manner described by White, Field, and Drinker (9). The rate of flow was determined by measuring the volume of lymph entering the cannula in an arbitrary time.

The methods of producing edema by plasmapheresis and by the limitation of protein in the diet have been described in a previous paper (10).[1] Edema fluid

[1] A slight modification has been made in the composition of the diet, that is, cod liver oil, 10 cc. daily, has been substituted for the butter fat. Animals maintained on the modified diet have remained in better health than those on the original diet and have exhibited fewer skin lesions. The cod liver oil was supplied through the courtesy of Mead Johnson and Co.

was obtained by puncturing with a needle into an edematous region and applying gentle pressure to the surrounding tissues. Most frequently this region was the loose tissue about the Achilles tendon of a hind leg. In several instances samples of ascitic fluid were obtained by puncture of the peritoneum. Samples of edema fluid and lymph were analyzed for total protein by a micro-Kjeldahl procedure which has been previously described (10). Whenever possible, samples of from 0.5 to 1.0 cc. were taken for analysis. The difference between duplicate determinations rarely exceeded 0.02 gm. per cent and was often less than 0.01 gm. per cent. Non-protein nitrogen was determined separately whenever the volume of sample permitted; in a few instances the figure obtained by analyzing blood serum collected at the same time was used in the calculation of protein from total determined nitrogen.

Lymph Flow in Normal Dogs

The data collected in these experiments are given in Table I.

The rates of lymph flow are expressed as cubic centimeters produced by the entire foot in 10 minutes, the calculation being the same as that employed by White, Field, and Drinker (9). It is based on the observation that a concentration of vessels occurs at the wrist and ankle, so that in both cases "there are three, or at the very most four, draining trunks." As there is no detectable difference in size one may assume that the flow through all is equal. A fair approximation of the flow of lymph from a part is therefore secured by multiplying the flow from a single vessel by three.[2]

The values recorded for each animal exhibit considerable variation. To appraise them properly several observations are pertinent. During periods of anesthesia or at other times of complete physical inactivity lymph flow ceases entirely. If a single movement occurs, or if the extremity is stroked, lymph at once rises in the cannula and may continue to rise at a declining speed for some seconds subsequently. Within a brief time the flow again ceases. The values recorded in Table I for sitting animals are, therefore, not a measure of lymph flow during physical inactivity but indicate merely that the sitting dog is rarely completely at rest. When, after a period of rest, lymph flow is stimulated by regular massage or motion, the first lymph always

[2] For those who may think that this method of expressing the rates of lymph flow involves too many assumptions, it is possible to regard the figures which express the volume of lymph draining from the entire foot in 10 minutes as representing more accurately the volume delivered by a single lymphatic trunk in 30 minutes.

TABLE I

Rate of Lymph Flow in Six Normal Dogs

Time	Anesthetic	Activity	Rate
Dog 10-00 (left fore leg)			
			cc.
2:15	N	Walking	0.9
2:45	N	Running	1.2
3:15	N	Sitting	0.6
3:45	N	Walking	0.7
4:15	N	Walking	0.7
4:45	N	Walking	0.7
5:15	N	Walking	1.2
Dog 9-2 (left hind leg)			
12:45	L	Massage	2.2
1:00	L	Massage	1.0
1:15	L	Massage	1.2
2:00	L	Massage	1.0
2:23	L	Walking	0.1
2:50	L	Walking	2.9
3:10	L	Walking	2.7
3:30	L	Walking	2.7
Dog 5-8 (right fore leg)			
2:00	E	Massage	11.7
3:15	E	Massage	9.5
4:00	N	Walking	5.2
4:30	N	Running	7.3
5:00	N	Sitting	0.5
5:30	N	Walking	4.8
Dog 1-31 (left hind leg)			
			cc.
11:25	L	Massage	—
12:25	L	Walking	1.8
12:55	L	Walking	1.6
1:25	L	Walking	1.6
2:05	L	Massage	— *
2:45	L	Walking	2.5
3:15	L	Walking	2.4
Dog 8-40 (right hind leg)			
10:45	L	Massage	4.4
11:15	L	Walking	1.6
11:45	L	Walking	1.7
12:15	L	Walking	1.4
12:45	L	Running	2.5
1:53	L	Massage	2.6
2:15	L	Walking	2.1
2:45	L	Walking	2.3
3:15	L	Walking	1.3
Dog 9-1 (left hind leg)			
2:45	L	Walking	0.8
3:15	L	Running	0.8
3:45	L	Walking	0.6

Lymph flow is expressed as total lymph from foot in 10 minutes. Lymph from single lymphatic trunk is multiplied by 3 to obtain total lymph. Collections were continuous throughout successive periods except as noted. Time, as entered in the table, refers to the median time during a period of collection. Thus, the rate of flow at 2:45 p.m. may be calculated from the volume collected between 2:30 and 3:00 p.m., or from the volume collected between 2:40 and 2:50 p.m., etc. Dog 8-40 rested between 1:00 and 1:45 p.m. while receiving 500 cc. of physiologic salt solution intravenously. Dog 9-2 rested between 2:40 and 3:10 p.m. while receiving 650 cc. of Locke's solution intravenously and Dog 1-31 rested between 1:40 and 2:05 p.m. while receiving 500 cc. of Locke's solution intravenously.

The following abbreviations are used in this and subsequent tables. E, ether anesthesia; B, nembutal [sodium-ethyl (*l*-methyl butyl) barbiturate] anesthesia; N, normal state after recovery from ether anesthesia; and L, local novocaine anesthesia.

* Observations during these intervals are omitted as the conditions of collection and measurement were altered. See Table II.

flows at a relatively rapid rate. As the stimulation is continued the rate decreases and finally attains approximately constant values. The maximum rates in Table I were measured immediately after periods of rest and were calculated from the volumes of lymph collected in the first 10 or 15 minutes. Table II records an experiment in which after a period of rest lymph flow was measured over a number of 15 second intervals. The short interval has accentuated the rapidity of the initial flow. A relatively constant rapid rate was main-

TABLE II

Rate of Lymph Flow during Successive Time Intervals Following a Period of Rest

Dog 1-31. Left hind leg. Local anesthesia.

Activity	Time interval	Volume of lymph collected	Rate of flow from foot
		cc.	*cc. per 10 min.*
None	25 min.	0	0
Massage	15 sec.	0.30	36.0
Massage	12 sec.	0.26	39.0
Massage	15 sec.	0.37	44.4
Massage	15 sec.	0.27	32.4
Massage	15 sec.	0.30	36.0
Massage	15 sec.	0.20	24.0
Massage	15 sec.	0.15	18.0
Massage	30 sec.	0.20	12.0
Massage	30 sec.	0.25	15.0
Massage	30 sec.	0.30	18.0
Massage	30 sec.	0.10	6 0
Massage	1 min.	0.20	6.0
Massage	2 min.	0.20	3.0
Walking	30 min.	2.50	2.5
Walking	30 min.	2.40	2.4

tained for 75 seconds only; thereafter, it declined progressively. In this case the initial rate of flow, approximately 38 cc. per 10 minutes, permits an estimate of the carrying capacity of the vessels and it is clear that this capacity greatly exceeds the normal rate of continuous flow. The fact will become significant when the findings with edematous dogs are considered. It is believed that the temporarily rapid initial flow depends on the quantity of extracapillary and intercellular fluid which has accumulated during the preceding period of

inactivity. With the commencement of activity this fluid is drawn at once into the lymphatics. In contrast the rates recorded after a constant flow has been established must measure the formation of fresh lymph. Such rates have regularly exhibited minor fluctuations but in general have been close to the minimum rates observed during activity. On this basis the rate of lymph production in the foot of a normal active dog can be estimated as 1.45 ± 1.20[3] cc. per 10 minutes. The figure represents the average of the minimum rates of flow in six dogs of this series and in eight dogs studied by White, Field, and Drinker (9).[4]

The data in Table I confirm the observation of White and his associates (9) that lymph flow varies with the degree of activity. The variation between flows produced by moderate and excessive activity (walking and running) is, however, small in comparison with the temporarily increased rate which follows complete rest.

Lymph Flow in Edematous Dogs

When edema is produced in dogs either by plasmapheresis or by dietary restriction, it rarely happens that all four extremities are involved simultaneously. For this reason it has been possible to measure lymph flow in edematous dogs from non-edematous extremities as well as from edematous extremities. With edematous extremities lymphatics have always been selected for cannulization which included the edematous part in the region drained. Because the toes and paws are more swollen in nutritional edema than in plasmapheresis edema, lymph from protein-fasted dogs drains more generally from edematous regions than that from animals treated by plasmapheresis. The rate of flow from seven edematous extremities in six dogs is shown in Table III. The average maximum rate of flow was 19.3 cc. in 10 minutes, a higher figure than was recorded with any normal animal. Individual measurements, however, exhibited much variation which in a rough way could be correlated with the degree of edema present. For example, the left hind leg of Dog 2-3 presented a mild edema and

[3] Standard deviation of the distribution.

[4] For the discussion which follows the reader should note that in speaking of the rate of lymph formation we shall be referring to data secured through a study of the minimum rates of lymph flow.

TABLE III

Rate of Lymph Flow in Edematous Dogs from Extremities Which Exhibited Edema

Time	Anesthetic	Activity	Rate
Dog 5-8 (right hind leg)			
			cc.
4:15	L	Massage	19.8
4:30	L	Walking	7.0
4:53	L	Walking	4.6
5:08	L	Massage	3.0
Dog 1-31 (right hind leg)			
12:33	L	Massage	30.0
12:40	L	Massage	7.8
1:00	L	Walking	2.7
1:22	L	Walking	2.5
2:00	L	Walking	2.7
2:30	L	Walking	2.6
3:00	L	Massage	2.5
3:30	L	Walking	1.6
Dog 9-1 (right hind leg)			
12:20	L	Massage	11.6
12:30	L	Massage	7.2
12:50	L	Walking	7.0
1:20	L	Walking	6.7
1:45	L	Walking	3.9
2:15	L	Walking	3.1
2:45	L	Walking	2.9
3:15	L	Walking	1.7
3:45	L	Walking	2.4
4:30	L	Walking	3.4
5:00	L	Walking	2.1
Dog 2-3 (left hind leg)			
			cc.
11:45	L	Massage	3.6
12:00	L	Massage	1.8
12:40	L	Walking	3.9
1:00	L	Walking	2.4
1:50	L	Walking	2.7
2:10	L	Walking	2.6
Dog 2-3 (right fore leg)			
12:20	L	Massage	49.2
12:25	L	Massage	17.4
12:30	L	Massage	18.6
12:45	L	Walking	5.3
1:05	L	Walking	3.8
1:50	L	Walking	3.2
2:10	L	Walking	3.9
Dog 8-40 (left hind leg)			
4:00	L	Massage	6.4
4:15	L	Massage	2.6
4:30	L	Massage	1.4
5:00	L	Walking	2.1
Dog 5 (right hind leg)			
3:00	L	Massage	14.3
3:20	L	Walking	7.1
3:40	L	Walking	5.4
4:20	L	Walking	5.3

In Dogs 5-8, 9-1, and 1-31 the edema resulted from plasmapheresis. In Dogs 5, 2-3, and 8-40 edema was due to protein starvation alone. Dogs 5 and 9-1 both received intravenous injections of 500 cc. physiologic salt solution; with the former this occurred between the 3:40 p.m. and 4:20 p.m. periods and with the latter between the 3:45 p.m. and 4:30 p.m. periods.

the maximum rate was 3.9 cc. in 10 minutes, whereas the right fore leg was markedly swollen with edema and yielded lymph at an initial rate of nearly 50 cc. per 10 minutes. As in the normal animal, the initial rate of flow was invariably greater than that subsequently recorded, the decline being greatest when the initial rate was highest. The rapid decline from the initial rate indicates that even in the edematous animal the lymphatics are able to carry fluid from the tissues at a faster rate than newly formed fluid can accumulate. The maximum rates of flow are probably not greater than those in normal animals during the first seconds following a period of rest and merely indicate the presence of an increased accumulation of extracapillary fluid (edema fluid) which is able to maintain an accelerated flow for a longer space of time. Table IV presents the observed rates of lymph flow in three edematous dogs from five extremities in which palpable edema was not demonstrable. An average maximum rate of flow of only 2.90 cc. per 10 minutes again exemplifies the dependence of initial flow on the degree of edema.

The minimum rate of flow, which provides an estimate of the rate of lymph formation, is interesting in both groups. With edematous extremities the average minimum rate was 2.57 cc. per 10 minutes and with non-edematous extremities it was 0.72 cc. per 10 minutes. Although a real difference between the groups may exist, the result suggests that observations on the edematous extremities may not have continued long enough to furnish a record of the true minimum rates.[5] In any case an average minimum rate for both groups together of 1.80 cc. per 10 minutes is not significantly different from that of 1.45 cc. per 10 minutes which was measured with normal dogs.

Effect of Stimulating Lymph Flow on Edema

The conclusion reached in the preceding section that during initial activity (massage and passive motion or normal walking) lymph drains from an extremity more rapidly than new lymph can be formed and that this flow depends upon previously accumulated extracapil-

[5] When the amount of edema is considerable several hours may elapse before constant rates of flow are established at minimum levels. With animals weakened by prolonged restriction of protein in the diet or by frequently repeated plasmapheresis operations, long continued physical activity is often impossible.

TABLE IV

Rate of Lymph Flow in Edematous Dogs from Extremities Which Were Non-Edematous

Time	Anesthetic	Activity	Rate
Dog 9-92 (right fore leg)			
			cc.
12:30	B	Massage	3.4
12:45	B	Massage	2.4
1:00	B	Massage	1.6
1:15	B	Massage	1.8
1:30	B	Massage	1.4
1:45	B	Massage	0.8
2:00	B	Massage	1.2
2:15	B	Massage	1.8
2:30	B	Massage	1.6
2:45	B	Massage	1.4
Dog 9-92 (left fore leg)			
1:15	B	Massage	2.6
1:30	B	Massage	1.4
1:45	B	Massage	1.2
2:00	B	Massage	0.6
2:15	B	Massage	0.6
2:30	B	Massage	2.0
2:45	B	Massage	0.6
Dog 6 (right fore leg)			
			cc.
2:00	B	Massage	2.8
2:15	B	Massage	1.6
2:30	B	Massage	1.1
2:45	B	Massage	1.8
3:00	B	Massage	3.7
3:15	B	Massage	4.0
3:30	B	Massage	6.4
Dog 6 (left fore leg)			
1:45	B	Massage	2.5
2:00	B	Massage	0.9
2:15	B	Massage	1.4
2:30	B	Massage	1.4
2:45	B	Massage	1.4
3:00	B	Massage	1.6
3:15	B	Massage	1.1
Dog 5-8 (left fore leg)			
2:45	L	Walking	3.2
3:15	L	Walking	2.5
3:45	L	Walking	1.5
4:30	L	Walking	0.5
5:15	L	Walking	0.3
6:05	L	Walking	0.9
7:05	L	Walking	0.2
7:45	L	Massage	0.5

Edema the result of plasmapheresis. With Dog 5-8 after initial 3 collections attempt was made to stimulate lymph flow by giving by gavage 10 gm. sodium chloride in 500 cc. water. Within 12 minutes diarrhea resulted and lymph flow decreased. A subsequent gavage with 500 cc. physiologic salt solution and an intravenous injection of 500 cc. physiologic salt solution were not attended by significant variation in the rate of lymph flow. The increased flow at the 6:05 p.m. period followed a previous rest period of 20 minutes.

lary fluid, is verified in a striking way when one observes the effect of such activity on the edematous state. Repeatedly it has been observed (Dogs 5, 5-8, 9-1, 8-40) that swelling and evidence of edema slowly disappear during these periods. With extremities carrying a cannula in a lymphatic trunk the decrease in clinical edema has always been correlated closely with the declining rate of lymph flow. In these cases a precisely similar decrease has always been seen in non-operated extremities and the phenomenon can, therefore, bear no relation to the operative procedure. Under such circumstances it is impossible to escape the conviction that edema fluid, that is, interstitial fluid, is being drained from the tissues through lymphatic channels. Finally, it may be noted that when edema is eliminated in the manner described it has always reformed after a subsequent overnight rest period.

Protein in Lymph from Normal and Edematous Dogs

In a recent monograph Drinker and Field (8) have brought together the observations of numerous investigators which show that lymph collected from a variety of animals under different conditions and in different regions always contains an appreciable and sometimes a considerable quantity of protein. They have tabulated fifteen of their own observations concerning leg lymph obtained from quiescent dogs under sodium barbital anesthesia. This lymph exhibited a protein concentration (refractometer) which varied between 0.70 and 5.71 gm. per cent and averaged 1.84 gm. per cent. The authors believe that the higher concentrations exist for short periods only and point to the experience of White, Field, and Drinker (9) who measured the protein content of lymph from non-anesthetized dogs during periods of normal activity. "When constancy of protein was reached in eight dogs, the concentrations were from 1.52 to 0.5 per cent of protein."

The results of our investigation (Table V) are in substantial agreement with those reported by the above authors. Among twelve normal dogs protein concentrations varying between 0.45 and 3.45 and averaging 1.59 gm. per cent were encountered. From five of the dogs specimens were collected during nembutal anesthesia. These showed an average of 2.06 gm. per cent of protein as contrasted with a figure of 1.25 gm. per cent from seven non-anesthetized dogs. The highest concentration observed in the absence of anesthesia was 1.89 gm. per cent. Table VI presents the concentrations of protein in the lymph from ten edematous dogs. The values range from 0.01 to 0.69 gm. per cent, average 0.23 gm. per cent, and are considerably lower

TABLE V

Proteins in Lymph and Serum of Normal Dogs

Dog No.	Anesthetic	Predominating activity	Protein of lymph		Protein of serum	Serum protein / Lymph protein
			Range	Average		
			per cent	*per cent*	*per cent*	
8-41	B	Massage	1.55–2.17	1.92	6.83	3.6
8-42	B	Massage	2.63–3.23	2.93	7.40	2.5
8-95	B	Massage	0.45–0.91	0.74	5.60	7.6
9-49	B	Massage	0.73–1.69	1.25	6.04	4.8
9-50	B	Massage	3.45	3.45	7.89	2.3
10-00	N	Walking	0.56–0.78	0.66	5.70	8.6
5-8	N	Walking	0.79–1.01	0.90	5.70	6.3
6-9	N	Walking	1.26	1.26	5.77	4.6
9-1	L	Walking	1.89	1.89	6.38	3.4
9-2	L	Walking	1.15–1.65	1.40	5.73	4.1
1-31	L	Walking	0.95–1.42	1.17	5.99	5.1
8-40	L	Walking	1.27–1.65	1.46	5.07	3.5
Average (nembutal anesthesia).....				2.06	6.75	
Average (without general anesthesia).....				1.25	5.76	
Average (combined).....				1.59	6.18	

TABLE VI

Proteins in Lymph and Serum of Edematous Dogs

Dog No.	Anesthetic	Predominating activity	Protein of lymph		Protein of serum	Serum protein / Lymph protein
			Range	Average		
			per cent	*per cent*	*per cent*	
9-92	B	Massage	0.06–0.07	0.07	2.10	30.0
6	B	Massage	0.15–0.69	0.39	2.60	6.7
5-8	L	Walking	0.05–0.25	0.11	2.49	22.6
8-40	L	Walking	0.11–0.14	0.13	2.88	22.2
9-1	L	Walking	0.34–0.60	0.46	3.32	7.2
2-3	L	Walking	0.12–0.25	0.17	3.38	19.9
5	L	Walking	0.18–0.24	0.22	3.24	14.7
1-31	L	Walking	0.01–0.08	0.04	3.20	80.0
8-38	Pm.*	Massage	0.29–0.53	0.37	3.00	8.1
8-06	Pm.*	Massage	0.23–0.60	0.36	3.00	8.3
Average.....				0.23	2.92	

* Lymph obtained post mortem.

than those in the lymph from normal dogs. A marked reduction in the proteins of serum has also occurred but inspection of the serum protein:lymph protein ratios indicates that the fall in lymph protein has occurred at a more rapid rate than the decline in serum protein. The ratio of average serum protein to average lymph protein in the normal dogs is 3.3; the same ratio in the edematous dogs is 12.7.

The Protein Content of Lymph and Edema Fluid

In comparing the composition of lymph with that of edema fluid it is important to bear in mind that the larger lymphatic trunks do not drain subcutaneous tissue exclusively. In part tributaries are received from joints, muscles, fascial planes, tendon sheaths, skin, and possibly bone. Among the portions of subcutaneous tissue drained by one vessel all degrees of edema may be present. Lymph collected from such a trunk cannot come exclusively from edematous subcutaneous tissue and it is unlikely that the various tributaries contribute equally to the total volume. Following cannulization the flow of lymph is first rapid and later relatively slow and the rapid flow is associated with a decrease in swelling in the edematous region. The fact suggests that the proportionate contributions into the main lymph channel are changing constantly during any period of collection. Finally, it is not unlikely that the continuous exercise necessary for maintaining a flow of lymph may of itself be the cause of variations in capillary permeability and so of fluctuations in composition of the lymph collected. In general the first specimens of lymph will contain a large volume of edema fluid and a small volume of fresh filtrate from the capillaries. Later specimens may be composed chiefly of newly formed filtrate.

In Table VII have been entered the protein concentrations of edema fluid and of the first lymph collected following cannulization. In six instances in which direct comparison is possible[6] (Dogs 5, 8-40, 2-3, 5-8, 1-31) the difference in protein concentration between the two fluids was from 0.02 to 0.09 gm. per cent and averaged 0.04 gm. per cent. The differences are small, as would be expected, although in

[6] Specimens from Dogs 8-06 and 8-38 are not compared as they were secured after death. Specimens from Dog 9-1 are also excluded as the edema fluid contained blood.

most instances they are outside the range of analytical error. It is interesting, then, to note that in four instances of the six the first lymph contained less protein than the corresponding edema fluid. The fact suggests that in some instances the protein in edema fluid may be increased above that in fresh filtrate from the capillaries by further diffusion of protein molecules from the blood during the period of dormancy in the tissues. The fluctuations in lymph protein during periods of continuous collection are presented graphically in Chart 1.

TABLE VII

Comparative Protein Contents of Edema Fluid and the Lymph Collected Immediately after Cannulization

Dog No.	Hind legs				Fore legs				Ascitic fluid	Nature of edema
	Right		Left		Right		Left			
	Edema fluid	Lymph	Edema fluid	Lymph	Edema fluid	Lymph	Edema fluid	Lymph		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
5	0.23	0.18								Nutritional
8-40	0.04		0.02	0.11					0.02	Nutritional
8-06	0.17	0.28	0.14	0.60		0.31		0.23	0.13	Nutritional
8-38	0.08	0.53		0.30		0.29			0.32	Nutritional
9-92						0.07		0.06	0.01	Plasmapheresis
6						0.32		0.15	0.03	Plasmapheresis
5-8	0.09	0.06	0.08							Plasmapheresis
9-1	0.86*	0.38	0.95*							Plasmapheresis
2-3			0.17	0.19	0.16	0.14				Nutritional
1-31	0.04	0.01	0.16*						0.01	Plasmapheresis

* These edema fluids contained blood. See text.

No dog in the group included in the chart produced either edema fluid or lymph the protein content of which exceeded 0.3 gm. per cent. The recorded fluctuations are, therefore, necessarily small. They are considerably less than those encountered in normal dogs. In four instances the lymph protein exhibited a progressive rise as collection was continued. In three of these (Dogs 5, 1-31, 8-40) the rise was slight; in one instance (Dog 5-8) it was strikingly rapid. With Dog 2-3 a different response occurred. Lymph from a hind leg declined progressively in protein content and lymph from a fore leg first in-

creased and then decreased in concentration of this substance. Comparison of these concentrations with those present in edema fluids from corresponding limbs shows that in five instances out of six the edema fluid protein lay within the range of variation of the lymph protein. In the sixth instance (Dog 8-40) lymph protein was constantly higher than edema fluid protein.

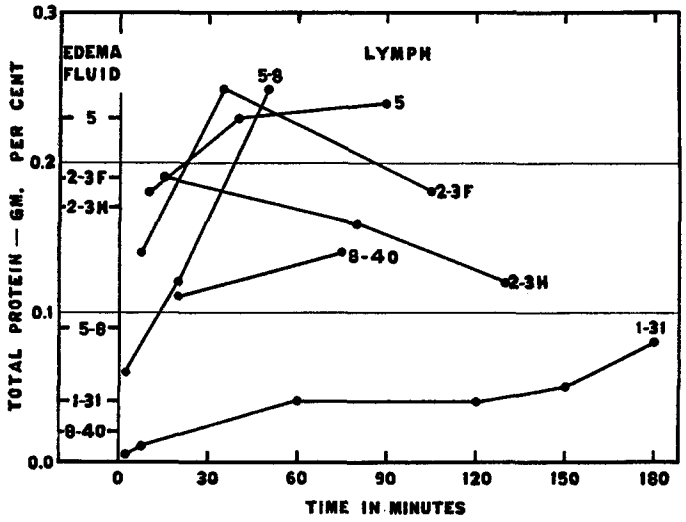

CHART 1. Protein concentration of edema fluid in relation to the fluctuating concentrations of protein in lymph during a period of continuous collection. The numbers identify particular animals, the letters *F* and *H* refer to fore leg and hind leg, respectively.

The Occurrence of Erythrocytes in Lymph

Systematic microscopic studies of the cellular elements in lymph have not formed a part of this investigation. Such studies have been made by Haynes and Field (11). The gross appearance of all specimens has, however, been noted as a routine; that is, they have been recorded as clear, or slightly, or moderately discolored with red blood cells. At first the possibility was entertained that these cells might be finding their way into the lymphatics through absorption from the tissues in the region of the operative incision. We have failed, however, to secure evidence in support of this possibility. In one experi-

ment the lymphatics of both fore legs were cannulated. On the right side no attempt was made to control hemorrhage during the operative manipulations and blood was purposely allowed to collect about the edges of the field. On the left side hemorrhage was controlled both by hemostats and with adrenalin. Lymph obtained from both limbs was entirely clear. In other experiments the operative field has been sopped with India ink or the ink has been injected subcutaneously in the same region before making the initial incision. In no case has the ink appeared in the lymphatics from which collection was being made. Although the majority of lymph specimens do not contain blood cells, their presence has been observed in samples collected both from normal and edematous dogs, both with and without general anesthesia, and when lymph flow was being stimulated either by massage, passive motion, or normal activity. We have come, therefore, to regard the finding as a normal physiologic phenomenon which, as such, may assist toward an understanding of the blood capillary → tissue space → lymphatic capillary circulation.

Summary of Experimental Observations

Lymph flow in normal dogs ceases entirely during periods of complete physical inactivity. During these periods capillary filtrate accumulates in the interstitial spaces and can enter the lymph channels at once when activity stimulates the pumping action of the lymphatic valves. The initial flow is, therefore, rapid but the rate declines quickly as the interstitial reservoir is emptied and finally becomes constant at a rate which corresponds to that at which new lymph is being produced. With the edematous dog the situation is similar but because the interstitial spaces contain more fluid (edema) the initial rapid flow can be maintained for a longer time than in the normal animal. Within 10 or 15 minutes, however, the rate of flow decreases and continued activity is accompanied by progressive and finally by complete loss of edema. The carrying capacity of the lymph vessels at all times greatly exceeds the rate at which new lymph can be formed. The data suggest that the rate of lymph formation, as estimated from the minimum rates of lymph flow, may increase slightly when edema is present. The increase, however, is surprisingly small and not beyond the limits of variations encountered in normal animals.

Lymph from the normal dog always contains an appreciable quantity of protein. Lymph from the edematous dog contains much less protein. The lymph protein deficit of edematous dogs is greater than can be accounted for on the basis of a proportionate loss corresponding to the serum protein deficit. The concentration of protein in lymph from edematous dogs is of the same order of magnitude as that of edema fluids although the two fluids are not identical in composition. Minor fluctuations in the protein of lymph occur while the collections are being made. The fluctuations may depend on varying proportionate rates of flow from different regions which send tributaries to the main lymph channels or they may result from variations in capillary permeability incident to the continuous exercise necessary for maintaining lymph flow.

Lymph from the lower leg of normal and edematous dogs sometimes contains red blood cells and sometimes it does not. Both increases and decreases in the number of erythrocytes may follow in succession as the conditions of collection are altered.

DISCUSSION

The theory of Starling (1) postulates that the exchange of fluid across the capillary wall is controlled by a balance between capillary blood pressure, which acts to force fluid from the capillary into the tissues, and colloid osmotic pressure of the blood plasma, which tends to draw fluid from the tissues into the capillary. The balance is adjusted so that mechanical pressure is greater than osmotic pressure at the arterial end of the capillary and less than osmotic pressure at the venous end. The gradient in pressure through the capillary leads to filtration of fluid at the arterial end and reabsorption of the fluid at the venous end. When filtration and absorption are equal, as is assumed in health, fluid, which is in effect an ultrafiltrate of plasma, circulates constantly through the tissues and so favors the free exchange of substance. The theory was elaborated in the experiments of Schade (4) on model capillaries made of collodion with which he demonstrated both filtration and reabsorption when the collodion tubes were perfused with serum under controlled pressure. In 1930 Landis (12) published the results of measurements on vessels at the base of the human finger nail. In a series of manometric determinations by a microinjection technique he showed an average fall in pressure from 32 mm. of mercury at the arterial end of capillaries to 12 mm. at the venous end. As the colloid osmotic pressure of the serum of human beings varies between 23 and 28 mm. of mercury, the conditions anticipated by Starling were realized in the measurements. From the quantitative standpoint information is still lacking regarding the volume of fluid which circulates through the tissue spaces as a

result of the pressure gradient in the capillaries. It has seemed to us, however, that this volume must be small in comparison with the movement of fluid which results when the flow of lymph is stimulated by physical exercise or massage.

In a previous communication (10) it was stressed that the movement of fluid across the capillary wall is controlled by forces on the outside as well as on the inside of the wall. The effective colloid osmotic pressure is the difference between the osmotic pressure of serum (S) and the osmotic pressure of the interstitial fluid (I), whereas the effective mechanical pressure is the difference between capillary blood pressure (C) and back pressure from the tissues (T). Under stationary conditions, that is at times when edema is neither forming nor receding, the forces are in a state of equilibrium expressed by the equation:

$$S - I = C - T$$

Recently Landis and Gibbon (13) have called attention to the necessity of considering tissue pressure in order to explain reasonably the diminishing rate of increase in arm volume following a given elevation in venous pressure.[7] In view of the findings in this investigation it becomes obvious that the state of equilibrium expressed by the above equation can be attained only during periods of complete rest; that is, at times when there is no perceptible flow of fluid through the lymph channels. During rest fluid accumulates in the subcutaneous tissue until the attending rise in pressure leads to a balance between the processes of filtration and reabsorption. That some extracapillary fluid does accumulate during rest even in normal animals under normal conditions of venous pressure may be assumed from the evanescent rapid flow of lymph which accompanies the onset of activity.[8] The

[7] Knowledge of the factors which govern tissue pressure is very inadequate. Recently Holland and Meyer (Holland, G., and Meyer, F., *Arch. exp. Path. u Pharmakol.*, 1932, **168,** 603) have presented measurements upon which they base the conclusion that marked edema is not attended by a disturbance of tissue pressure. In the discussion which follows we are concerned with immediate variations in pressure which may be assumed to follow changes in lymphatic activity and not with the question of remote changes which might be conceived to result from the operation of forces responsible for edema.

[8] From observations through a transparent chamber in the rabbit's ear, Clark and Clark (Clark, E. R., and Clark, E. L., *Am. J. Anat.*, 1933, **52,** 273) have concluded that under usual conditions no free fluid is present in the tissue spaces. If

efficiency of the lymphatic pump must be considerable for, as we have seen, markedly edematous areas can be reduced to a non-edematous state within several hours. The removal of tissue fluid must lead to an immediate fall in pressure in the tissue spaces and disturb the relation between capillary filtration and reabsorption in such a way as to increase the quantity of fluid actually leaving the capillaries. That is to say, the drop in pressure will lead to an increase in the area of capillary wall functioning for filtration and to a decrease in the surface available for reabsorption.

Under these circumstances it is pertinent to inquire whether during physical activity the process of capillary reabsorption may not be completely in abeyance and the whole of the capillary wall function for filtration. That such may be the case is suggested by the failure to observe any considerable increase in the rate of lymph formation in animals exhibiting serum protein deficits. The Starling theory demands that a declining osmotic pressure of the plasma be accompanied by a steadily increasing area of capillary wall available for filtration. For this reason alone an accelerated lymph formation should be expected in the presence of hypoproteinemia. The failure to measure such an increase may, therefore, be taken to suggest that the whole of the permeable surface of the capillary already functions for filtration during periods of physical activity in the normal animal. Furthermore, regardless of explanation, the finding also suggests that failure of the mechanism for reabsorption plays a greater rôle in the causation of edema than increase in the rate of filtration.

The equation given on page 79 also expresses the fact that the effective colloid osmotic pressure of the serum is represented by the difference between the total osmotic pressure of serum and the osmotic pressure of the fluid on the outside of the wall of the capillary. If the wall of the capillary were almost impermeable to colloids, as was believed by Starling (1), Schade (4), Krogh (3), and others, the fluid outside the wall would contain so little protein that its effective osmotic pressure would be negligible. At the present time, however, the extensive evidence assembled by Drinker and Field (8) is too convincing to allow continuance

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their observations should be shown to hold for the subcutaneous tissues in general, it would become necessary to think that the fluid which accumulates outside the capillaries during rest is located within the lymphatic capillaries and not strictly speaking in the tissue spaces.

of belief in the impermeability of all capillaries at all times to protein. Krogh, Landis, and Turner (14) have pointed out that the summation of the processes of filtration and reabsorption may mean that the filtering surface of the capillary is bathed in a fluid of low protein content whereas at the absorbing surface the concentration may be raised considerably. They suggest that lymph may represent the fluid remaining after the process of reabsorption is complete and are unwilling to look upon it as representative in composition of average tissue fluid. According to them the experience of several authors with mechanical filtration edemas indicates that extravascular fluid often contains less than 0.1 per cent protein and they believe that this figure represents more closely the composition of capillary filtrate. We have seen, however, that lymph often contains more than 1 per cent protein. Aside from the fact that it is difficult to believe that bordering layers of fluid in the tissue spaces can differ so markedly in composition, it may be noted that reasons have already been given for supposing the process of capillary reabsorption to be in complete abeyance during periods of active functioning of the lymphatics. Under such conditions White, Field, and Drinker (9) found that the composition of lymph became constant between levels of 0.5 to 1.52 per cent of protein. Our experience has been similar. It might be reasoned, then, that these figures provide a closer approximation of the composition of capillary filtrate in normal dogs at least. In a previous communication, however, the present authors (15) have given another reason for being unwilling to accept as proven the identity of tissue fluid and lymph. It was possible to think that only occasional capillary loops permitted the passage of protein, that this protein did not diffuse generally into surrounding structural units, but rather that it passed at once into the nearest lymphatic radicle. As there was reason to believe that the volume of filtrate would be greater from those capillaries which allowed protein to pass than from those which did not, and, as the lymph collected from a large trunk represented a mixture of the streams coming from many different radicles, it appeared evident that this lymph might be representative neither of average tissue fluid nor of average lymph in the many different radicles. As yet no completely satisfactory method of testing the validity of this theory has been devised. Nevertheless, the observations on the occurrence of erythrocytes in lymph strongly support the belief that occasional capillaries may exhibit an unusual degree of permeability. In fact, Drinker and Field (8) have stated that "the red-cell content of lymph is probably often due to rupture of blood capillaries into lymph capillaries." If such gross rupture is possible, and, as we have seen, the phenomenon is not infrequent, it is not unreasonable to think that dilated pores of a size sufficient to permit the passage of protein may be even more frequent. It is obvious that the principle involved in this theory may still hold even if the capillaries are everywhere somewhat permeable to protein. It does, however, imply that permeability and rate of filtration may vary between different capillaries in such a way that lymph collected from a larger trunk fails to be representative in composition of either capillary filtrate or tissue fluid.

The finding in this investigation that the protein deficit of lymph from edema-

tous animals is too great to be accounted for on the basis of hypoproteinemia alone, has suggested two possible explanations: (1) Either as a result of hypoproteinemia or because of distention of the tissue spaces with edema, the permeability of the capillaries for protein may be lessened. (2) The fall in plasma osmotic pressure may so increase the filtering area of capillary wall and decrease the resorptive area that the reduction in lymph protein is due not only to the decrease in plasma protein but also to a further dilution with water which in the normal animal would be reabsorbed into the capillaries. An assumption of diminished permeability of the capillaries has to us been the more acceptable explanation for the reason that an increase in the filtering area of capillary wall would be expected to increase the volume of capillary filtrate, and we have seen that the rate of lymph formation in edematous animals does not give evidence of such an increase.

Comparative analyses of lymph and edema fluid in this investigation have revealed an approximate identity in the composition of the two fluids. The finding lends some support to the belief that lymph may be regarded as representative in composition of average tissue fluid. Under the circumstances it is perhaps most wise not to stress slight differences in composition which were, however, outside of the range of analytical accuracy. Nevertheless, to our minds, the differences have been of such a nature as to emphasize the manner in which transient changes in capillary permeability (as may occur during exercise) or variations in the quantity of lymph coming from different structural units in the tissues (as certainly occurs when the supply of free edema fluid has been exhausted) are reflected in immediate fluctuations in the quantity of protein in lymph. Under such circumstances the data do not as yet warrant the conclusion that lymph and tissue fluid from normal animals will agree as closely in composition as lymph and edema fluid from animals with serum protein deficits.

SUMMARY

1. The experimental observations have been summarized at the end of an earlier section. The more important facts only will be recapitulated here.

The capacity of the lymphatics for removing fluid from the tissues greatly exceeds the rate at which freshly formed tissue fluid can be made available for removal. Edematous regions can be rendered non-edematous by the application of measures, such as massage, passive motion, or normal exercise, which activate the lymphatics.

During continuous activity the rate of lymph flow is first variable and later relatively constant. Constant rates of flow must correspond to the production of fresh lymph. A study of the constant rates indicates that lymph formation in the edematous animal is certainly only slightly greater, and possibly not greater at all, than under conditions of normality.

When the protein of plasma decreases, the protein of lymph is also lowered. The loss of protein from lymph takes place at a faster rate than from plasma, so that the ratio of serum protein to lymph protein is greater in the edematous than in the normal animal.

In edematous animals the concentration of protein in lymph is of the same order of magnitude as the concentration in edema fluids. The two fluids are not, however, identical in composition. Minor fluctuations in the protein content of lymph always occur during a period of continuous collection.

2. The factors involved in the circulation and accumulation of tissue fluid are discussed. Reasons are given for offering the following suggestions.

Significant differences in tissue pressure or tension exist between the states resulting from quiescence and activation of the lymphatics. The differences give rise to variations in the relative areas of capillary wall, functioning for filtration and reabsorption. When the lymphatics are activated it is possible that capillary reabsorption may be completely in abeyance.

A decline in the proteins of plasma may be associated with a diminished permeability of the capillaries. Such a lowering of capillary permeability would account for two features, both of which have been demonstrated: (1) failure to observe an appreciably increased rate of lymph formation in the edematous animal, and (2) the extremely low concentration of protein in lymph from edematous animals.

Although the difference between the protein concentrations of edema fluid and lymph from the same region is small, the conclusion is not yet justified that a similarly small difference exists between normal tissue fluid and normal lymph.

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