THE CIRCULATION OF BODY FLUIDS IN THE FROG.

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In the course of experiments dealing with the capillary circulation of the frog it was discovered that the fluid in the subcutaneous lymph spaces normally contains 0·29 to 2·17 p.c. of protein. As we were concerned with questions relative to capillary permeability it seemed of immediate importance to reach a clear understanding of this exchange of protein substances through the capillary walls. Fortunately the solution of the question has been aided by the recently published figures of directly measured capillary pressures in the frog mesentery by Landis (1), and by a volumetric estimation of the rate of total fluid exchange between the blood and tissues by Isayama (2). To these facts we now add determinations of the protein content and the colloid osmotic pressure of the blood and lymph; and endeavour to combine these lines of evidence in a discussion of the physical and chemical forces which produce a constant and normal movement of the blood plasma with a large fraction of its proteins from the blood vessels into the lymphatic circulation.

Methods.

The frogs used in the following determinations were chiefly *Rana temporaria*, although a few individuals of *R. esculenta* are included. There appeared no significant difference between species or sexes.

In the majority of cases the lymph was collected from the lymph sac which lies dorsal to the tendon of the gastrocnemius. In a few instances it was taken from the other subcutaneous lymph spaces, and in those specimens used for osmotic pressure determinations fluid from the peritoneal cavity was added to obtain the necessary volume. From pharmacological evidence Straub (3) considers that there is a free intercommunication of the lymph spaces with each other and with the peritoneal cavity, so that practically we have to do with one large lymph space.

The analyses were carried out between March 9 and April 19 and therefore include individuals taken before the breeding season with

immature eggs in the ovaries; a number of individuals during the height of the breeding season including some markedly œdematous copulating males, and specimens of both sexes which had apparently returned to a normal condition after the completion of egg-laying. It is not the purpose of this paper to discuss the cause or significance of the spontaneous œdema which occurs particularly in the males during the activity of the breeding season, although measurements made on such individuals are so specified in the tabulation. The close correspondence between our figures and those of the colloid osmotic pressure of the three plasma specimens reported by White(4) for a different species at a different season of the year make us feel that it is extremely unlikely that the phenomena we are describing are peculiar to the spring breeding period. It is possible, however, that the absolute values given in our tables will prove to be somewhat below figures which may be obtained for normally feeding frogs that are not undergoing the depletion in body protein which may attend the later stages of egg and sperm production.

The protein quantitation was accomplished by the use of a Zeiss Eintauch-Refraktometer (Pulfrich), using Prism 1. The necessary correction factor for the non-protein constituents of the plasma was obtained from the ultra-filtrate of a specimen of frog lymph. The scale reading of this ultra-filtrate was 18·4 which indicates an nD 1·33450. This corresponds very accurately with the nD of Ringer's solution made with the composition given by Clark(5). The nD of 1 p.c. of frog-lymph protein was found to be 0·00240 by the analysis of a specimen of lymph with a known refractometer scale reading[1].

The osmotic pressure determinations were carried out according to the method of Krogh(6). Both the blood and lymph specimens were allowed to clot and the centrifuged serum employed in the analyses.

Results.

The determinations are expressed in the form of the three following tables:

[1] 3 c.c. of lymph weighing 3·025 grm. were diluted to a volume of 50 c.c. with water. A 20 c.c. portion of this dilution was heated and the nitrogen content of the resulting coagulum determined by Kjeldahl analysis was 0·0025 grm. Assuming that the coagulum represents the total protein, and using the factor 6·45 for the conversion of weight nitrogen to weight protein, 100 c.c. of lymph (100·83 grm.) contains 1·34 grm. protein. The per cent. of protein by weight is therefore 1·33.

In using this value it is assumed that the heat coagulum represents the total protein content of the specimen. A second 20 c.c. portion of the same dilution contained 0·00334 grm. total nitrogen as determined by Kjeldahl analysis.

TABLE I.

Frequency distribution of the percentages of protein in the fluid from the lymph sacs of 60 normal frogs.

p.c.	No. of individuals
0·29 to 0·69	9
0·74 to 1·35	38
1·38 to 2·17	13

TABLE II.

Percentages of protein in the fluid from the lymph sacs of seven frogs with the marked œdema which accompanies the mating season.

	p.c.		p.c.
Frog	1 0·60	Frog	5 1·65
	2 1·07		6 1·73
	3 1·38		7 1·78
	4 1·57		

TABLE III.

Colloid osmotic pressures of blood and lymph.
(Including determinations by Krogh, 1922; and White, 1924.)

	Blood			Lymph		
Description	Osmotic press. mm. water	Protein p.c.	*O.P.* prot. p.c.	Osmotic press. mm. water	Protein p.c.	*O.P.* prot. p.c.
1. Normal male	69·0	2·87	24·0	37·0	1·50	24·6
2. Normal male	68·0	2·49	27·3	28·0	1·22	22·9
3. Normal male*	134·0	4·29	31·2	77·0	2·17	35·4
4. Emaciated female	15·0	1·39	10·8	5·0	0·43	11·6
5. Mixed 4 frogs	69·0	—	—	—	—	—
6. Œdematous male	54·5	2·01	27·0	28·5	1·07	26·6
7. Œdematous male	66·0	2·61	25·2	47·0	1·77	26·5
8. Œdematous male	76·0	2·80	27·1	37·0	1·37	25·0
9. Mixed 4 œdematous males	86·0	3·27	26·3	77·0	1·60	48·1
Krogh, 1922						
Frog's blood hirudinised	55·0	2·1	26·0	—	—	—
"	60·0	1·5	40·0	—	—	—
White, 1924 (oxalated)	mm. plasma					
Frog 1	96·0	2·4	40·0	—	—	—
Frog 2	98·0	2·52	39·0	—	—	—
Frog 3	115·0	2.8	41·0	—	—	—

* It is to be noted that of the 60 frogs included in Table I this animal showed the highest percentage of protein in the lymph.

DISCUSSION.

In presenting his figures for the osmotic pressure of the blood colloids of the frog, White(4) points out the forces which might bring about filtration through the glomerular capillaries. The subsequent measure-

ments reported by Hayman(7) which show glomerular capillary pressures higher than the ones of Hill which are cited by White add further evidence that this filtration may occur. Landis(1) by a method of direct measurement has found that the average pressure in the arteriolar capillaries of the frog mesentery is 14·5 cm. of water, and that of the venous capillaries is 10·0 cm. of water. Using the figures of White this author points out that capillary pressure may be above or below the osmotic pressure of the plasma colloids throughout the whole length of any capillary in successive moments. As our figures are lower than those given by White, they make it more safe to conclude that there exists a filtration of fluid throughout the systemic system to the extent that it may be assumed that the capillary pressures of the mesentery hold for other regions of the body. In making such an assumption it is important to recall the fact that the frog possesses two portal systems, that of the liver and that of the kidney. Both of these organs impose the obstruction of a second capillary bed in corresponding portions of the venous system. The liver receives the venous blood of the intestinal tract including the rectum and bladder as well as blood from the Bulbus cordis, from the abdominal wall and a part of that of the hind legs. The kidney receives the remainder and larger fraction of the blood from the hind limbs, as well as blood from the dorsal part of the trunk and the rump and in the female from the oviduct (8). It is almost certain that the existence of a secondary capillary bed must act as an important factor in the determination of the pressure gradient in the vessels of the primary circuit.

Another factor acting to increase the volume of filtration is the presence of 0·29 to 2·17 p.c. of protein in the fluid of the lymph spaces. From this finding we must infer a degree of permeability to protein on the part of the walls of adjacent capillaries which is entirely unlike that commonly assumed to exist in mammals except in certain tissues such as the liver. It is our impression that this increased permeability is a characteristic of the vessels of the skin of the frog. The filtration of fluid will naturally be increased wherever the effectiveness of the already low intravascular colloid osmotic pressure is reduced by this permeability of the capillary wall to protein. Thus the average osmotic pressure of the colloidal proteins of the lymph has been found to be 42 mm. of water and the difference between this value and that of the intravascular 71 of the blood colloids is but 29 mm. of water representing the effective colloid osmotic pressure tending to hold fluid within the capillary. Opposed to this, at least in the mesentery, is a capillary pressure averaging 122 mm. of water.

It is of interest therefore to find that Isayama(2) working from an entirely different point of view has estimated that in the toad a volume of fluid equal to that of the total blood plasma passes from the vessels into the tissues and is collected by the lymphatic system at least fifty times in twenty-four hours. This writer estimated changes in blood volume by counting the erythrocytes and determined the amount of blood volume diminution during the first ten minutes following the destruction of the lymph hearts.

The physiological significance of the actively beating lymph hearts of the frog at once becomes obvious if we consider that we are dealing with an animal which is carrying on a general filtration of fluid from its systemic circulation, and in addition possesses at least a large capillary area which is freely permeable to the protein constituents of the blood plasma.

SUMMARY.

The protein content of normal frog lymph varies from 0·29 to 2·17 p.c., the usual value being 1·00 p.c. The average osmotic pressure of the colloids of the blood serum of nine frogs was 71 mm. of water. In the same individuals the average colloid osmotic pressure of the lymph was 42 mm. of water.

These values considered in the light of known capillary pressures show physical and chemical forces acting to produce a normal movement of blood plasma including a fraction of its protein from the blood vessels into the lymphatic system. This is consistent with published volumetric estimates of the rate of fluid exchange in the frog.

Such a concept is of the utmost importance in experimentation involving the use of the frog for the study of capillary permeability.

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