THE HISTOLOGY OF LYMPH, WITH SPECIAL REFERENCE TO PLATELETS

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The primary object of this investigation is to determine whether blood-platelets occur in lymph. The material employed consists of stained smear preparations of lymph from the thoracic duct of the dog, treated according to Wright's method for blood smears. The desirability for further studies on the structure of lymph arises by reason of the discrepancies in the results reported by recent investigators. Schäfer ('12) makes the unqualified statement that "both lymph and chyle contain thrombocytes" (p. 394). Davis and Carlson ('09) in their study of the cell-content of lymph, make no mention of platelets, permitting the inference that they did not see these elements in lymph. Vinci and Chistoni ('10), on the basis of careful microscopic studies of the lymph, of dog, cat, and rabbit, conclude that lymph lacks platelets (p. 209). Howell ('14) comes to the conclusion that "platelets do not occur in lymph from the thoracic duct of the dog."

The following general facts would lead one to expect to find platelets in lymph: 1. Platelets play an important part in the clotting of blood "by liberating an additional supply of thromboplastic substance and of prothrombin" (Howell, '14). Lymph, in common with blood, contains fibrinogen and thrombin, and under similarly favorable conditions clots promptly. Since thrombus formation in blood is apparently largely dependent upon the presence of platelets, the latter would be expected to occur also in lymph, which has a similar physical and chemical constitution and clots like blood under similar conditions. 2. With the possible exception of platelets, lymph contains the same cellular elements, in vastly different proportions, as blood. If any bar-

rier between the blood-vascular and the lymph-vascular systems can be passed by all the other cellular elements of the blood, even the erythroplastids, it seems reasonable to suppose that platelets also would not be excluded from lymph. 3. The origin of blood-platelets from segmenting pseudopods, and from fragmenting larger areas of cytoplasm, of a certain type of megakaryocyte in the red bone-marrow is well established (Wright, '10; Bunting, '09; Downey, '13; Jordan, '18). Since the platelets pass readily into blood-capillaries of the marrow, no plausible explanation presents itself as to why they are excluded from the lymphatics of the marrow.

The recent work of Howell ('14) is the most thoroughgoing that has yet been devoted to the question of platelets in lymph. It is required, therefore, that this work be first carefully analyzed, with a view to determining whether any possible contingency remains which has not been fully met in this search for the possibly very elusive platelets in lymph. Such a contingency would serve as the only excuse for any addition to an otherwise apparently conclusive research.

Howell's investigation of platelets in lymph is part of a larger research relating to the mechanism of coagulation in blood and lymph. He obtained the lymph of his experiments from dogs anesthetized with morphia and ether, by placing a vaselined cannula into the thoracic duct. The rate of flow was variable, but averaged about 0.5 cc. per minute. The lymph from dogs starved for forty-eight hours clotted in from ten to twenty minutes. The more milky lymph from fed animals clotted in from thirty to sixty minutes in the case of earlier specimens; lymph collected after an hour or two only clotted after from one to three hours. Howell searched for platelets both in the fresh and in oxalated lymph of these experiments. He examined also the corpuscular sediment obtained by centrifugalization of oxalated lymph, but neither in the deposit from a first nor in that of a second centrifugalization could he discover platelets, though the same method applied to blood-plasma revealed them in great abundance. Howell ('14) concludes that "platelets do not constitute a normal element in the lymph."

According to Howell, clotting involves the coöperation of four constituents: 1, fibrinogen, in solution in plasma and lymph; 2, prothrombin, liberated by platelets and by lymphocytes; 3, antithrombin, present in lymph and in blood-plasma in substantially equal amounts, and 4, thromboplastin, liberated by platelets and lymphocytes and tissue cells in general, and operating to neutralize the antithrombin. Pure lymph uncontaminated with tissue juices is said to coagulate more or less imperfectly compared with mammalian blood, but it coagulated promptly and firmly if tissue extract is added to it. Howell explains the inferior clotting of lymph as due to the comparative paucity of thromboplastic material, the result of the absence of the less stable platelets and the consequent restriction of its source to the relatively more stable lymphocytes. This explanation is in accord with the earlier conclusion of Vinci and Chistoni ('10) that coagulation can occur in the complete absence of platelets. These authors concluded, moreover, that "the principal morphological blood element intimately related to the phenomenon of coagulation is the white corpuscle" (p. 212).

In view of the facts that general considerations relating to the comparative microscopic structure of lymph and blood, and to the mechanism of clot formation as formulated by Howell, seemed to favor the probability that lymph contains platelets as expressly claimed by Schäfer ('12), it occurred to me that failure on the part of Howell, and of Vinci and Chistoni, to discover the alleged elements might be due to some unfavorable factor in their technical procedure with reference to platelets. It is well known that the leucocytes in the blood-vessels tend to collect peripherally. Also the extreme adhesiveness of the platelets and leucocytes in general is obvious. It seemed possible that the slow flow of the lymph (0.5 cc. per minute, Howell) might permit the elimination of platelets from specimens collected by cannula from the thoracic duct, by reason of their adhesion to the wall of the duct. A final link in the chain of apparently complete evidence respecting the absence of platelets in lymph as given by Howell, seems to demand an examination of lymph in the form of specially prepared smears made directly from the thoracic duct.

Such hypothetical demand furnished the reason for the present investigation and suggested the special technic employed.

This investigation includes lymph smears from two different dogs prepared at an interval of several weeks. Control specimens of blood were made from these same animals. The technical procedure, for assistance in which I am greatly indebted to Dr. J. A. Waddel, professor of Pharmacology, at the University of Virginia, was as follows: The first dog was quickly killed with ether. The thoracic and abdominal cavities were immediately opened, and a segment of the thoracic duct tied off and excised. The segment was thoroughly rinsed in physiologic salt solution at body temperature, to cleanse it of any possibly adherent blood. A cut was promptly made into this segment of thoracic duct and the lymph content allowed to flow onto slides. The lymph had a clear, watery appearance. Subsequently the segment was cut along the entire length and slides were smeared from the inner surface of the duct. The slides were at once treated according to Wright's staining technic. Control blood smears, stained according to the same method at the same time, showed abundant platelets. But the lymph smears apparently lacked platelets completely.

I was at first surprised to find erythroplastids among the cellular content of the lymph. I suspected that their presence was the result of the death struggle, they having possibly been sucked back from the subclavian vein. However, if their presence was to be accounted for on this basis, typical platelets should occur for the same reason. I find that Howell, and Davis and Carlson, also record the presence of a certain small number of red corpuscles in lymph. In the second experiment almost immediate death was caused by administration of chloroform by mouth, with the idea of obviating any possible reverse flow from the subclavian vein at death. But the preparations from this specimen also showed the presence of a few red corpuscles. Accordingly, we may conclude, I believe, that erythroplastids are a normal constituent of lymph. Nor did any of this second series of slides reveal typical platelets.

The question arises as to how the presence of the erythroplastids in lymph is to be interpreted. Davis and Carlson ('09) only regard the presence of erythroplastids as due to blood admixture (p. 16). In accord with the monophyletic theory of blood-cell origin, such red corpuscles might represent differentiations of certain lymphocytes, serving as hemoblasts, in their slow passage through the lymphatic tree. However, if this conclusion were correct, one should probably expect to find also erythroblast stages; but such could not be detected in my slides. It seems perhaps more reasonable to conclude that the few erythroplastids present in lymph have their origin in the red marrow drained by certain perivascular lymphatic terminals of the system. This conclusion, however, requires that a consistent answer be given to the question as to why platelets, which theoretically could enter the system in the same way, cannot be found in lymph from the thoracic duct. It seems altogether probable that platelets do actually enter the marrow lymphatics in small numbers. The lymph of the thoracic duct contains elements generally liberated by platelets, namely, prothrombin and the thromboplastin (Howell), but in relatively smaller amounts than found in blood-plasma. Platelets are notoriously unstable structures. In their relatively slow passage (compared with the blood circulation) through the lymphatics to the thoracic duct, the relatively small number that may have entered the system may have entirely disintegrated, meanwhile contributing the relatively small amount of prothrombin and thromboplastin characteristic of lymph; to which the lymphocytes under favorable conditions may contribute a certain additional amount, as suggested by Howell. Considering the origin of the bulk of the lymph from the plasma via the tissue spaces, and the continual disintegration of blood-platelets, the presence of these elements (thrombin and thromboplastin) in lymph can be accounted for at least in part on the basis of this relationship between blood plasma and lymph. Similarly with respect to the thromboplastin of the tissue cells.

Starling ('15) inclines to regard blood-platelets as precipitation products in plasma, following contact with foreign bodies or the lowering of its temperature from 37°C. to 18° (p. 837). He

bases his opinion on the observations, following Buckmaster: 1. When a film of blood, held in a platinum loop and kept at body temperature, is examined under the microscope, no platelets can be seen; on cooling, platelets make their appearance. 2. When noncoagulable plasma, e.g., peptone plasma and oxalate plasma, is kept for twenty-four hours at 0°C., after removal of all formed elements by centrifugalization, a precipitate forms 'indistinguishable from blood-platelets.'

With regard to the first observation it may be justly urged that the fact that the platelets are not microscopically visible in the platinum-loop preparations at body temperature is not conclusive proof that such are not actually present. The refractive index of the platelet protoplasm at body temperature may be so close to that of blood-plasma as to render the platelets practically invisible; on cooling, the refractive index of the platelets may change to a point where they become visible against the background of the fluid plasma.

In opposition to the deductions from the second set of observations, stand the results of the present investigation, namely, that in the smear preparations (essentially of lymph which was allowed to cool and thus suffered coagulation and precipitation of certain proteids) certain platelet-like bodies formed which at first appeared indistinguishable from genuine blood-platelets. However, a careful study, as described in detail below, showed that these bodies are quite different structures, essentially compound precipitation products. Moreover, considering the chemical and physical similarity between blood-plasma and lymph, and in large part their common origin, it seems strange that genuine platelets should precipitate only from the former and not from the latter. And in addition, the fact should again be emphasized that the original results of Wright ('10) regarding the megakaryocyte origin of platelets have been confirmed by at least three subsequent independent workers (Bunting, Downey, Jordan) and his conclusions may therefore confidently be regarded as firmly established. Continued skepticism regarding the genesis of platelets as first described by Wright can only rest, it seems to me, on a failure to repeat in detail Wright's technic in the study of red marrow.

In view of Starling's interpretation of platelets as precipitation products, it becomes important to give in detail the steps by which I reached the conclusion, on the basis of my microscopic preparations, that the lymph from the thoracic duct of the dog lacks platelets completely. The slides were made a year ago and were at once carefully studied. Numerous structures were seen which simulated platelets. The possibility seemed to present itself that these bodies were modified or disintegrating platelets. The investigation was temporarily abandoned with the idea that the material at hand did not permit of definite conclusions. I subsequently again studied the slides, and again arrived at a point of uncertainty. A third, more intensive effort, has brought me to certain and definite conclusions: Platelets do not occur in lymph of the thoracic duct; the half-dozen bodies in my preparations regarding which some doubt may remain do not materially alter the precision and generality of this conclusion; various and numerous precipitation products appear which simulate platelets, but which are clearly of different origin and of compound structure. This material accordingly yields additional and significant evidence for the disproof of the conclusion that platelet-like bodies in peptone and oxalate plasmas are genuine platelets.

The stained smear preparations of lymph have a background of a homogeneous or very finely granular vacuolated substratum, ranging in color, depending upon a varying density, from a faint pinkish-blue to a light lilac color. Among the spherical vacuoles may occasionally be seen small spheroidal or oval areas of more condensed substratum. Throughout this substratum are scattered the lymph cells. These are much more abundant in the specimens from the first dog, a difference depending, as Howell has shown, upon nutritive conditions, that is, whether the animal was starved or recently fed. The small lymphocytes predominate, forming at least 90 per cent of the entire cell-content. Large lymphocytes are also relatively abundant; similarly large mononuclear leukocytes with reniform lilac-colored nucleus, and a wider shell of faintly pink cytoplasm. Occasional neutrophils, eosinophils, and erythroplastids also occur, probably of marrow

and tissue-space origin. Occasional endothelial cells, detached from the duct wall in the process of making the smears, also occur. The large mononuclear leukocytes are in the vast majority of cases distorted or fragmented. These cells are evidently much more adhesive and fragile, both in respect to cytoplasm and nucleus, than the small lymphocytes. In the close vicinity of such broken leucocytes clumps of fibrin-fibrils occur, suggesting that these cells are more favorable regions for thrombus formation.

Scattered throughout the substratum are also innumerable spheroidal and bacillary lilac-colored metachromatic granules, most probably proteid in chemical nature. Such frequently collect in small groups and may thus simulate platelets. The resemblance is especially close where several such granules have collected within one of the minute oval coagula above described. It is only when constant reference is made to the platelets of the control blood-preparations that precise differences are established. In this work, whenever such a platelet-like body was encountered, reference was immediately made to the blood smears. The following are the essential criteria finally established for definitely distinguishing between such platelet-simulacra in lymph and genuine platelets of blood: The blood-platelets, like their simulacra in lymph, vary greatly in size; the size extremes of platelets are in the proportion of at least 1 to 10 volumes; but true platelets are very frequently collected into groups; their lymph simulacra are generally scattered and never in large groups. The peripheral hyaline layer of blood-platelets is very pale blue, the central granules violet, after Wright's stain; in the lymph simulacra the corresponding parts are pinkish-blue or light lilac, with deep lilac or violet granules. Moreover, the granules in the case of the lymph structures are coarser and less regular (spheroidal and bacillary) than those of true platelets.

The inference may perhaps be justly drawn on the basis of these comparative observations on lymph and blood, with respect to platelets, that the so-called 'platelets' of peptone and oxalate plasmas, on which is founded the precipitate-explanation of platelets (Buckmaster; Starling), are homologous with the platelet-simulacra of lymph. The evidence seems conclusive that

platelets are preformed structural elements of blood—not precipitated products—and that they do not form a normal constituent of lymph of the thoracic duct. This may mean only that the possibly small quota contributed to the peripheral portion of the lymphatic tree in relation to red bone-marrow, from which the relatively smaller amount of prothrombin and thromboplastic substance of lymph may have a partial source, suffered disintegration during their relatively slow progress towards the thoracic duct, and accordingly do consequently not appear as structural elements among the blood-cell content of thoracic-duct lymph.

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