THE HEMORRHAGIC AND HEMOPOIETIC NODULES IN THE ALAR AND INTERFEMORAL MEMBRANES OF PIZONYX VIVESI (CHIROPTERA)[1]

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TWENTY-TWO FIGURES

In the wing membrane of the bat *Pizonyx vivesi* (Menegaux, '01) there are characteristic nodular areas which have been described as "glandular masses" (Miller, '06, '07). The presence of these masses, unique among vespertilionid bats, is a characteristic of this genus and its sole species. Our interest in circulatory phenomena in bats' wings and in the comparative morphology of *Pizonyx,* prompted us to investigate the nature and significance of these nodular masses.

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MATERIAL AND METHODS

Twenty-three specimens of *Pizonyx vivesi* were examined grossly. These had been collected at the following localities: (1) Patos Island (north of Tiburón Island) — 9 specimens, (2) Isla Partida (near Ángel de la Guardia Island) — 9, (3) Puerto Refugio, Ángel de la Guardia Island — 5; all of these localities are in the Gulf of California. One of the above

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specimens from Puerto Refugio and another from Turtle Bay, one-half mile offshore from the cannery village on the western coast of Baja California, were studied microscopically.

The specimens were presumably fixed in 10% formalin by the collectors, and were later transferred to ethanol solutions for storage.

Microscopic studies of the nodular areas were made in whole mounts of large areas of the wing and interfemoral membranes, and in serial sections of 10 μ thickness. In the preparation of permanent whole mounts, areas of wing and interfemoral membranes were excised and pinned out in shallow dishes. The following staining procedures were used on the whole mounts: (1) Ehrlich's acid hematoxylin (diluted 1:9 water) for 9 hours; (2) Ehrlich's acid hematoxylin (diluted), followed by Lillie's ('48) buffered azure eosinate; (3) Ehrlich's acid hematoxylin (diluted), followed by Mallory's triple connective tissue stain; (4) acidified azure-eosinate; (5) van Gieson's mixture; and (6) Heidenhain's iron hematoxylin (Bensley and Bensley, '38), both with and without eosin counterstain. The hematoxylin and azure components of these stains were the most useful in showing structures within the membranes. After dehydration through a series of ethanol solutions of increasing strength, the stained pieces of membranes were washed in xylene and mounted in balsam.

The serial sections of nodular masses from various places in the wing and interfemoral membranes were stained by the following procedures: (1) Ehrlich's acid hematoxylin and eosin; (2) Heidenhain's iron hematoxylin (Bensley and Bensley, '38); (3) Heidenhain's modification of Mallory's connective tissue stain (Azan) (Bensley and Bensley, '38); (4) Mallory's phosphotungstic acid hematoxylin (Lillie, '48); (5) Lillie's ('48) reticulum technic; (6) Azure II-eosinate (Lillie, '48) (two to 6 hours), (a) without other stains, (b) preceded by 11 hours in orcein, (c) preceded by Ehrlich's acid hematoxylin (3 min.) and followed by eosin (3 min.).

LOCATION AND GROSS MORPHOLOGY

The nodules are evident on gross examination as thickened and darkened areas. Their locations in both wings and interfemoral membranes of the 23 grossly examined specimens are shown in plate 1. The nodules were found in the basal part of the interfemoral membrane (uropatagium) and in most areas of the wing membrane except the propatagium. Miller ('06, '07), however, mentioned their occurrence only near the middle of the forearm. The fact that the nodular structures in other areas of the wing and interfemoral membranes are similar histologically was demonstrated by comparison of serial sections of nodules from these different areas. Although nodules were found in all specimens examined, they are not entirely consistent in their location, either in the two wings of any one specimen or in the wings of different specimens. It was found, nevertheless, that there are three rather sharply defined places in the plagiopatagium where the nodules occur most frequently. These three primary nodular areas are shown and numbered in plate 1. In area 1 nodules were present in 96% (44/46) of the wings examined; in area 2, in 63% (29/46); and in area 3, in 57% (26/46). The location of the nodules frequently coincides with the location of major intersections of elastic fiber bundles and skeletal muscle fiber bundles within the membrane. The location and relations of these fiber bundles in one area 2 nodule are shown in plate 4, figure 5.

The 20 females and three males comprising the series of grossly examined specimens do not show any sexual differences in location or size of their nodules. Inasmuch as all of the specimens were collected during March ('36, '37, '51), it is not yet possible to assess the possibility of seasonal variations.

The size and shape of the nodules vary, but are to a certain extent characteristic of the area in which they occur. Likewise the variability of the size and shape is characteristic of the particular areas of occurrence. For example: The largest nodules (up to 15×6 mm) occur in area 1; these

tend to be oblong and highly variable in size and shape. However, their longest axes tend to parallel the bands of skeletal muscle and associated blood vessels which run from the distal anterior quadrant to the proximal posterior quadrant of the wing. Sometimes in area 1 there are two or three parallel, separate or partially fused nodules in the same wing. In area 2 the nodules are large or moderate in size (up to 5×5 and 6×4 mm) and tend to be elliptical or nearly circular. In area 3 the nodules are usually small (up to 5×1 mm) and oblong, but are sometimes elliptical or nearly circular. Their long axes run in the same direction as those of area 1 nodules.

HISTOLOGY

The histology of the wing and interfemoral membranes of bats has been described by Schöbl (1871), Leboucq (1897, 1899), Morra (1899), Toldt ('07), Sabussow ('10), Ackert ('14), Schumacher ('31, '32a, b), and the blood and lymph vessels of the membranes have been studied by Jones (1852, 1868), Luchsinger (1881), Grosser ('01), Karfunkel ('05), Mislin ('41a, b, '47, '48), Webb and Nicoll ('44), Nicoll and Webb ('46), Mislin and Kaufmann ('47), Champy, Demay and Laurel ('47), and others. The following account concerns the modifications observed in the nodules of *Pizonyx*. For more general aspects of wing and interfemoral membrane morphology the reader is referred to the above references.

Contrary to Miller's ('06, '07) original supposition, the *Pizonyx* nodules are not "glandular masses," and should not be confused with the truly glandular organs found in the wings of *Saccopteryx* and *Emballonura*. An excellent review of the glandular organs has been provided by Schaffer ('40). Although sebaceous and sudoriferous glands occur in the *Pizonyx* nodules, these glands are no larger, nor are they noticeably more abundant than in adjacent unmodified areas of the membranes. On the contrary, the nodular masses are composed primarily of extravasated blood and hemopoietic tissue.

The peripheral portions of the nodular areas in the membranes usually exhibit a broad zone of connective tissue impregnated with extravasated blood cells. Smaller areas of extravasated blood occur more centrally in the nodules and also outside the peripheral zone of extravasation. It is clear that the hemorrhages did not result from injuries sustained at the time of capture or of death or from post mortem changes, since all stages of red blood cell disintegration and phagocytic activity may be observed in different parts of the areas of hemorrhage. Phagocytosis in these areas is seen first in the extravasated monocytes, which remain rounded in outline but become engorged with brown granules. In areas of apparent chronic extravasation, large phagocytes derived from either or both connective tissue cells and endothelial cells are seen (plate 5, fig. 10). These cells at first have the same shape, relation and nuclear structure as their unmodified counterparts, but they later become more rounded, are engorged with erythrocytes and their degeneration products, and finally disintegrate, leaving a large, dense deposit of brown pigment granules.

In the peripheral zone of extravasation it is usually difficult to relate the phenomenon to any particular vessel, but in the central area of the nodules where there are small loci of extravasated cells, these frequently can be related exactly to their vessels of origin. In these instances, there are observed dilated sinusoidal vessels, with definite breaks in the simple endothelium. The endothelial layer appears distended and is the only distinct layer present in the walls of the vessels. Extravasated blood cells are observed in the loose connective tissue in the region of the break in the vessel wall. In areas of most recent hemorrhage, as indicated by degree of degeneration, the extravascular blood cell types are in the same relative numbers as they are in arterial blood. Sinusoidal vessels lined only by a simple endothelium and surrounded by loose connective tissue swollen by extravasated blood cells are found in the peripheral zone of extravasation, and breaks in the walls of these vessels are sometimes seen.

It seems probable that much of the hemorrhage occurs by rhexis rather than by diapedesis.

In the part of the zone of extravasation closest to the center of each nodule, there is a transitional zone where the hemorrhaged red blood cells are scarcely recognizable, but remain as irregular eosinophilic masses. Between these masses and penetrating the walls of the adjacent sinuses there are many amoeboid wandering cells (plate 5, fig. 14). Similar irregular eosinophilic masses with wandering cells form loci scattered within the nodules. Within these loci the vessel of origin may be seen as either (1) a collapsed and occluded remnant, in which the endothelial cells have become large, and pale, resembling primitive reticular cells, or as (2) a venous sinus lined with a simple endothelium and minimal delicate connective tissue fibers. Thin plate-like areas of similar irregular eosinophilic masses occur immediately beneath the basement membrane of the dorsal epidermis of the nodules and appear to represent hemorrhages in the capillaries supplying the epidermis. In these subepidermal eosinophilic masses, amoeboid wandering cells are likewise found. Capillary beds other than those under the dorsal epidermis have small areas of hemorrhage within the nodules. These other hemorrhagic capillary beds will be considered later, with the histological regions to which they belong.

In some of the subepidermal plates and in certain loci within the nodules where irregular eosinophilic masses are present, these masses, presenting a coarse fibrous network, are stained a very dark brownish-red with the phosphotungstic acid hematoxylin. Since they also are stained pink to pale red by hematoxylin and eosin or azure-eosin technique and pale orange to brilliant red by Mallory's (Azan) connective tissue stain, these masses probably contain fibrin. These staining reactions and the morphology of the network-like structures agree with those described for fibrin by Lillie ('48).

The analysis of the histological modifications in the nodular areas should at this point be divided into two parts. One

of these concerns the influence on the dorsal epidermis and the connective tissue immediately beneath it. The other concerns the effects on the connective tissue stroma of the nodular areas. All of the changes observed in these two areas appear to be the products of a series of events, the first of which, the extravasation of blood, is the same in all areas. The subsequent histological changes differ in the two areas because certain of the tissues and their activities are different.

Cutaneous modifications

In the dorsal epidermis the local hemorrhages in the subepidermal capillaries cause the formation of plate-like areas of massed blood cells and fibrin. Amoeboid wandering cells then penetrate this clot as well as the epidermal layers immediately above. Along with the amoeboid wandering cells there soon appear other cell types; particularly evident are neutrophils and eosinophils. The inflammatory response in these areas varies in degree. In many cases the overlying epidermis is completely disrupted by the penetrating leucocytes, so that an absolute gap or eroded area is formed in the epidermis (plates 2, 4, fig. 4). During the inflammatory massing and penetration of leucocytes into the epidermis and its subjacent connective tissue, the epidermal cells increase greatly in size and number. This epidermal hypertrophy and hyperplasia is most pronounced at the circumference of the eroded areas. Within each eroded area the densely massed leucocytes and a faintly granular and fibrous intercellular material are exposed to the atmosphere. This clump of massed leucocytes extends from the exposed surface to near the center of the connective tissue of the membrane. Around the deeper margins there are irregular patches of clotted erythrocytes and fibrin, originating from capillaries in these areas. Following the inflammatory response and consequent disruption of epidermis and connective tissue, three constructive processes are evident: (1) epidermal proliferation reducing the gap of the wound, (2) cicatrization within the clumps of leucocytes and amorphous

intercellular material, and (3) penetration of the clumps by capillaries. The epidermis eventually heals over the eroded area, as the most superficial part of the exposed connective tissue becomes closely knitted with heavy horizontal collagenous fibers stretching from one side of the wound to the other. Meanwhile the dense clumps of cells and intercellular material are modified, becoming looser, more fibrous, and less cellular. Formerly eroded areas can still be detected after complete healing due to the fact that an unusually dense feltwork of heavy horizontal collagenous fibers remains in the connective tissue immediately below the dorsal epidermis.

While the ventral epidermis of the membranes remains thin and without subepidermal hemorrhages, the dorsal epidermis undergoes hypertrophy and hyperplasia, as has already been noted. The ventral epidermis of both nodular and unmodified areas and the dorsal epidermis of unmodified areas shows only a thin stratum corneum and a stratum germinativum of only one or two cells in thickness. In the dorsal epidermis of the nodular areas, the stratum corneum is thicker and lies over a distinct but very thin stratum lucidum, which rests on a stratum granulosum, usually of but one cell in thickness. This overlies a stratum germinativum containing an outer zone of greatly flattened eosinophilic cells, from two to 8 cells in thickness, and an inner zone of polyhedral or cuboidal germinative cells, commonly 4 to 5 cells in thickness. Mitotic figures are common in the germinative cells of the dorsal epidermis of the nodular areas and rare in the corresponding cells of the ventral epidermis generally and the dorsal epidermis of unmodified areas.

Connective tissue modifications

In the connective tissue of the nodular areas the most complex modifications are found. Within the nodules the bands of elastic fibers and skeletal muscle fibers and the accompanying major arteries and veins lie close to the ventral epidermis. The immediately adjacent connective tissue remains relatively slightly modified. However, the connective tissue

dorsal to the main vessels and fiber bundles is greatly thickened and modified in a series of histological changes, all stages of which may be seen within any of the larger nodular areas. The first two of these changes, extravasation and subsequent phagocytic activity and invasion by leucocytes, have already been described.

Following the invasion of the connective tissue by leucocytes, the array of cell types found becomes more complex and varies greatly from one small area to the next. This appears to be due to (1) certain of the cells giving rise to blood cell precursors and other cell types related to blood cell formation, and (2) local variation in the degree and direction of cellular specialization, particularly hemopoietic specialization. Thus, in some areas there are scattered leucocytes, phagocytes, fibroblasts and few if any hemopoietic elements (plate 4, fig. 7); in other areas the most prevalent cell types are neutrophil and eosinophil myelocytes (plate 4, fig. 8) or erythroblasts and normoblasts (plate 4, fig. 9). Eosinophils and neutrophils are the cells most abundantly formed in the hemopoietic areas. Aside from basophils, none of which were found, erythrocytes are the blood cells least commonly formed in the hemopoietic areas. Furthermore, while most, if not nearly all, the leucocytes formed in these areas appear normal, many and probably most of the erythrocytes and normoblasts appear abnormal. Microcytes, abnormally small normoblasts (plate 5, fig. 11), and normoblasts with atypical nuclear structure (plate 4, fig. 6) are common. Many of the latter at least appear to have resulted from atypical or arrested mitosis.

Unsuccessful attempts were made to find some correlation between type of hemopoietic activity and kind of relationship to blood vessels and other histological features. Nevertheless, it is apparent that hemopoietic activity in general is associated with connective tissue areas whose vascular supply has been repeatedly disrupted by hemorrhages. However, the factors contributing to the biochemical milieu inciting the hemopoietic activity are not known. Moreover, it is not

known with any certainty which cells respond to the hemopoietic stimuli. From careful examination of the cell types and their intermediates in the blood-forming areas, three possible origins are suggested: (1) Certain of the lymphoid cells having arrived in the area by extravasation or in response to it, may revert or give rise to cells of hemocytoblastic potentialities. (2) The endothelial cells of occluded or isolated capillary segments appear to revert to a large pale reticular type of cell, which may not degenerate but divides to form cells resembling hemocytoblasts. (3) Certain of the connective tissue cells of fibroblastic potentialities and appearance may revert to or give rise to cells of hemocytoblastic potentialities. In the above three possibilities, either myeloblast or lymphoblast can be substituted for hemocytoblast, since dependable criteria for distinguishing these in our material could not be found. Certainly the histological picture demonstrable with the techniques used in this study does not conclusively exclude any of the above possibilities, but it does appear to favor them in the order in which they have been stated.

Blood and lymph vessels

Arterial, venous, and lymphatic vessels are found within the modified connective tissue areas of the nodules. Since it is the dorsal part of the connective tissue of the membranes that is modified in the nodules, the vessels here are dorsal branches of the main vessels lying near the skeletal muscle and elastic fiber bundles. Blood enters this specialized connective tissue by arterioles which branch from arteries lying more ventrally. The arterioles subdivide and lead into capillaries. The arterial vessels of the nodules appear unmodified, but the venous and lymphatic vessels of the hemopoietic connective tissue areas of the nodules are frequently different from their normal counterparts.

The capillaries of these areas are connected either with venules of normal caliber or with greatly dilated venous sinuses (plates 2, 3). Both of these vessel types are thin-walled and do not appear to have smooth muscle cells. They

often have a thin, fibrous adventitia, however. Leucocytes pass into these vessels not only via capillaries but also by penetration of the vessel walls. In the slides of the nodules, leucocytes are commonly seen with extended pseudopodia passing into or through the walls of these vessels. This is not true for the larger venules and veins. The thin-walled venules and venous sinuses of the nodule connective tissue can perhaps more accurately be considered as venous capillaries. They are irregular in course and diameter and are not always accompanied by arterioles (plate 3).

It is uncertain whether the circulation in the nodule connective tissue is entirely closed. Between the true capillaries and the thin-walled venous capillaries, particularly those dilated ones forming venous sinuses, there remains the possibility of an open circulation through the hemopoietic tissue.

Lymphatic sinuses are present in the modified connective tissue of the nodules (plate 3). The lymphatic sinuses are usually greatly dilated and have walls that are so thin that they are not distinguishable from the reticular hemopoietic connective tissue surrounding them. For this reason, only the dilated portions of the lymphatics are readily observed. The tracing of the courses of these vessels was not possible in serial sections, since collapsed or occluded segments of the lymphatics were so difficult to distinguish from the surrounding tissue. Although lymphocytes and monocytes are the most abundant cells within the lymphatic sinuses, myelocytes are also common. The subsequent fate of these myelocytes is uncertain; however, they presumably pass into the surrounding hemopoietic tissue.

HEMATOLOGY

A comparison of the relative numbers of blood cells of different types in arterial and venous vessels in an area 2 nodule is provided in table 1. It should be noted particularly that: (1) the percentage of the blood cells which are leucocytes is 0.3% in arterial vessels and averages 17% in venous vessels, and (2) the relative abundance of the types of leuco-

cytes is different in the arterial and venous vessels. Although blood cell counts were made only in the vessels of an area 2 nodule, the results are not believed to differ significantly from what occurs in nodules in other areas. This conclu-

TABLE 1

Blood cell counts and per cents in arterial and venous blood vessels of an area 2 nodule. The arterial blood cell counts were made from the blood contained in 8 vessels (arterioles and arteries), some within the nodular area and some just outside of it. No differences could be detected in the counts or per cents from different types of arterial vessel. Therefore the arterial cell counts were combined. The venous blood cell counts were made from the blood contained in 8 vessels (venules and veins), all within and draining part of the nodular area. Since differences were noted in the blood cell per cents of different venous vessels, the data for each vessel is given separately (1–8) and also summarized (S). N = *number of cells counted.* % L = *per cent of leucocytes.* % T = *per cent of total.*

		VENOUS BLOOD									ARTERIAL BLOOD
		1	2	3	4	5	6	7	8	S	S
Erythrocytes	N	53	564	304	181	183	390	662	1109	3446	7924
Lymphocytes	N	1	9	9	9	1	4	21	27	81	14
	% L	..	16	17	14	..	11	10	10	12	54
Monocytes	N	1	9	12	19	1	12	46	55	155	8
	% L	..	16	24	29	..	34	22	21	22	31
Phagocytes	N	0	0	0	1	0	0	0	0	1	0
	% L	0	0	0	2	0	0	0	0	..	0
Neutrophil Myelocytes	N	0	0	2	0	0	3	24	5	34	0
	% L	0	0	4	0	0	9	11	2	5	0
Neutrophils	N	1	6	4	4	0	9	45	44	113	3
	% L	..	10	8	6	0	26	21	16	16	11
Eosinophil Myelocytes	N	0	0	2	4	0	3	14	36	59	0
	% L	0	0	4	6	0	9	7	13	8	0
Eosinophils	N	3	33	22	28	5	4	62	101	258	1
	% L	..	58	43	43	..	11	29	38	37	4
Total leucocytes	N	6	57	51	65	7	35	212	268	701	26
	% T	10	9	14	26	4	8	24	20	17	0.3
Total cells	N	59	621	355	246	190	425	874	1377	4147	7950

TABLE 2

Comparison of leucocyte sizes in the venous blood of Pizonyx nodules (hematoxylin azure-eosinate stain) with those in the general circulation of three other genera of bats. N = *number of cells measured.* $\bar{x}$ = *mean diameter.* Q_1 *and* Q_3 = *first and third quartiles.* Min. and Max. = *minimum and maximum measurements*

	LYMPHOCYTES				MONOCYTES				NEUTROPHILS				EOSINOPHILS			
	N	$\bar{x}$	Q_3 Q_1	Max. Min.	N	$\bar{x}$	Q_3 Q_1	Max. Min.	N	$\bar{x}$	Q_3 Q_1	Max. Min.	N	$\bar{x}$	Q_3 Q_1	Max. Min.
Pizonyx vivesi	50	3.4	3.8 3.1	4.4 1.9	50	4.7	5.0 4.3	5.8 3.4	50	5.3	5.8 4.9	6.9 4.3	50	4.9	5.0 4.6	6.1 3.6
Tadarida brasiliensis Geoffroi. (Martinez, '39a)	..	8.16	9.13 7.20	.. ..	..	15.05	16.67 13.44	.. ..	..	10.48	11.28 9.69	.. ..	..	12.59	13.47 11.70	.. ..
Desmodus rotundus murinus Wagner. (Martinez, '39b)	100	7.19	8.03 6.30	.. ..	100	13.69	14.60 12.77	.. ..	100	9.98	10.70 9.26	.. ..	10	10.89	11.70 10.29	.. ..
Artibeus J. jamaicensis Leach. (Martinez, '41)	329	7.86	8.75 6.97	.. ..	16	15.37	16.85 13.89	0 ..	313	9.60	10.30 8.91	.. ..	24	12.82	14.56 11.08	.. ..

sion is based upon qualitative examination of the blood cell content of vessels in nodules from several of the areas.

In the study of the blood cell types in the tissues and vessels of the nodular areas, the following observations were made (see plate 5).

The erythrocytes, practically all of extrinsic origin, are circular, biconcave discs (plate 5, fig. 16). The thickness at the center of the disc is 0.3 μ or less; the greatest thickness of the rim of the disc is 1.4–2.0 μ. The average diameter of 100 measured erythrocytes is 4.6 μ (3.6–5.5). The average diameters of erythrocytes of 13 species of bats reported in the literature, range from 4.35 to 6.8 μ (Worth, '32; Peters, '32; Isaacs, '38; Knoll, '39; Martinez, '39a, b, '41). Of these species only *Tadarida brasiliensis* has red blood cells of a size range comparable to that of *Pizonyx*. In *Myotis,* to which genus *Pizonyx* is said to be nearly related (Miller and Allen, '28) the average erythrocyte diameter ranges from 6.0 to 6.8 μ in the three species *(myotis, daubentoni,* and *lucifugus)* for which data have been published.

The leucocytes studied in the nodular areas probably originated predominantly from the hemopoietic tissue of the nodules. Measurements of the free cells in venous vessels of nodular areas show that each leucocyte type is much smaller here than it is reported to be in the blood of certain other genera of bats (table 2). To what extent the small size of the leucocytes in the nodular area veins is due to their special origin is not known. Study of the blood cells in the general circulation would determine this.

Lymphocytes and monocytes of the nodule veins are comparatively uniform and typical. Within the surrounding tissue, however, they become modified, certainly into phagocytes and probably also into hemocytoblasts and fibroblasts.

Intravascular neutrophils are variable, particularly in their cytoplasmic staining reaction. Some are nearly clear, some pale blue, and some pink or very faintly granular. The nucleus is two-lobed in 4%, three-lobed in 32%, 4-lobed in 56%, and 5-lobed in 8% of the neutrophils in the nodular veins.

Intravascular eosinophils are similar and vary primarily in density of cytoplasmic granulation and number of nuclear lobes. The eosinophilic cytoplasmic granules are spheres 0.2–0.3 μ in diameter. The nucleus is not lobed in 4%, two-lobed in 36%, three-lobed in 56%, and 4-lobed in 4% of the cells in the nodular veins.

CYTOLOGY

Certain of the tissue cell types in the nodules are of special interest and deserve further comment.

Mast cells are common in the connective tissue of the membranes within the nodular areas as well as outside of them. They are frequently most concentrated along blood vessels. Within the nodular areas mast cells of all stages of development, maturation and disintegration were found. The early mast cell is smaller, 5–10 μ in diameter, has a smaller and more darkly staining nucleus, and proportionately less cytoplasm, which contains basophilic granules of variable size (plate 5, fig. 12). In later mast cells the size of the nucleus and the amount of the cytoplasm increase greatly. The basophilic granules increase in size and, at least in fixed material, fuse to form a coarse network throughout the cytoplasm. The nucleus becomes large and pale. The shape of the cell frequently becomes irregular and the nucleus becomes oblong or oval. In the most advanced mast cells the basophilic reticulum becomes exceptionally coarse and its strands, although of irregular thickness, do not appear to be formed of individual granules. In disintegrating mast cells, the coarse basophilic reticulum breaks down in some areas of the cytoplasm, but remains in others and the surrounding medium becomes hyaline. The reticulum appears to be somewhat firm and to support the cell membrane in these disintegrating cells, although the cell membrane may be lost in some areas and the nucleus shriveled and misshapen.

Megakaryocytes are present but not common in the hemopoietic tissue of the nodules. They are usually very large, but sizes down to 10 μ in diameter are found. A typical example is shown in plate 5, figure 20. The cytoplasm in mega-

karyocytes of all sizes is nearly lilac in azure-eosinate preparations. In the smallest cells it tends to be more basophilic, however. In the smallest cells, usually nearly oval in shape, the cytoplasm is faintly granular, only slightly flocculent, and nonvesicular. In larger megakaryocytes, the cytoplasm becomes flocculent and highly vesicular, and the cells have long and irregular extensions into the surrounding tissue. It could not be determined whether these were involved in platelet formation. The nuclear material in the megakaryocytes always stained solidly black and either formed an irregularly lobulated mass near the center of the cell or was composed of separate chromatin masses connected to one another to a variable extent by dark threads. In a very few megakaryocytes the chromatin masses were dispersed around the periphery of the cell. The peculiar nature of the cytoplasm and the cell shape of the megakaryocytes always distinguished them from other large cells undergoing mitosis. In the latter, reticulo-endothelial or primitive connective tissue cells, the cytoplasm remains very pale or nearly hyaline and only faintly, if at all, granular.

DISCUSSION

It is generally accepted that under normal physiologic conditions in adult mammals the formation of the myeloid elements is confined to the bone marrow, and that in various abnormal conditions extramedullary or ectopic myelopoiesis may occur (Maximow and Bloom, '48). One wonders whether the hemopoietic nodules of the membranes of *Pizonyx* are the result of abnormal conditions, since they occur in all individuals and generally in specific places. Local extramedullary myelopoiesis has been observed in a great variety of organs, generally as a result of experimental or pathological conditions (Doan, '38; Lang, '38). In the *Pizonyx* nodules the hemopoiesis seems to be definitely related to chronic hemorrhage and inflammation of the connective tissue. Chronic circulatory disturbances or inflammation have been found to be sometimes associated with extramedullary myelopoiesis

(Latta, '21; Mandelstamm, '24; Ssyssojew, '26). It is believed by some that the specific erythrocytogenic factor resides primarily in a relatively high concentration of carbon dioxide in the regions of the hemopoietic tissues (Jordan and Speidel, '24; Jordan, '26); however, the fundamental stimulus for erythropoiesis in the adult mammal is generally considered to be anoxia (Grant and Root, '52).

The possibility that the wing and interfemoral nodules are a response to an increased systemic need for blood cells or to a pathology or unusual condition of the bone marrow, could not be determined since studies of the other blood-forming organs were not made. It is hoped that such studies may be made in the future.

Whatever the fundamental stimulus or stimuli to hemopoiesis may be in the *Pizonyx* nodules, the primary factor in the etiology of the nodules appears to be chronic hemorrhage and inflammation. There is no evidence that these effects are caused by local parasitism or infection. It remains possible, however, that a general systemic condition which weakens the blood vessel walls causing extravasation and consequently inflammation, is responsible. The fact that the nodules usually occur where the skeletal muscle and elastic fiber bands of the membranes form major intersections, suggests that the vessel walls are exposed to stresses here that caused their breakdown. Since it is known that: (1) a very slight negative vitamin C balance may produce capillary fragility, particularly in areas of friction (Dalldorf, '38; Reid, '43), and (2) *Pizonyx* subsists on fish and arthropods (Burt, '32; Walker, '50; Reeder and Norris, in press), it is suggested that this bat may have a mild vitamin C deficiency. The other bats which are fish-eating (Noctilio) or appear to be adapted for it *(Rickettia)* do not possess nodular masses in the wing or interfemoral membranes (Miller, '07; Allen, '36). However, this could be due to a less deficient diet, a decreased need for vitamin C, or some protective adaptation.

It is possible that the engorgement of the vascular plexus in the membranes induced by overheating (Cowles, '47;

Reeder and Cowles, '51) may be a contributing factor to vessel breakdown and extravasation. It may also be the chronic factor which maintains extravasation within the nodular areas and stimulates the hemopoietic activity.

The dilated venous capillaries which are partly responsible for the chronic extravasation in the nodules suggest that the back pressure in the veins may help to maintain blood stasis and edema in the nodular connective tissue. A study of the blood flow in these vessels in living specimens should prove interesting.

The bat's wing has been used in studies of living vessels. It should also be useful in studies of hemopoiesis in living tissue. The possibility of experimental production of hemopoietic tissue in bats' wing membranes, and subsequent studies of the relations of blood flow to hemopoiesis, should not be neglected.

SUMMARY

1. In the wing and interfemoral membranes of the bat *Pizonyx vivesi* (Menegaux) there are, characteristically, nodules which are the sites of chronic hemorrhage, inflammation and hemopoiesis, rather than of glandular activity.

2. The nodules have been observed in all areas of the membranes except the propatagium, but are most consistently located in three specific areas of the plagiopatagium. They usually occur at the major intersections of skeletal muscle and elastic fiber bundles.

3. The primary factor in producing the nodules is extravasation of blood cells; this may be due to a weakening of the vessel walls by, (A) mild vitamin C deficiency, (B) greater stress placed on vessels at the intersections of muscle and elastic fiber bundles.

4. Possibly active factors in increasing blood volume and extravasation within the nodules are, (A) back pressure in the veins, and (B) engorgement of the vessels during excessive heating.

5. Extravasation of blood cells occurs by rhexis and possibly by diapedesis. It occurs from capillaries within and around the nodular areas and from dilated venous capillaries or sinuses within the hemopoietic tissue of the nodules.

6. The dorsal epidermis and the dorsal part of the connective tissue of the membranes are the tissues affected in the nodular areas. In both regions, following hemorrhage, phagocytosis of erythrocytes, formation of fibrin-like material, and inflammatory changes occur. When they occur immediately beneath the dorsal epidermis, leucocytes penetrate and disrupt the epidermal layers, and the epidermal cells undergo hypertrophy and hyperplasia. When they occur in deeper areas, they lead to hemopoietic activity.

7. Hemopoiesis in the nodules is thought to be centered in one or some combination of the following cells: (A) extravasated or amoeboid lymphoid cells, (B) reverted endothelial cells of occluded or isolated capillary segments, (C) reverted connective tissue cells of fibroblastic potentialities and appearance.

8. Both leucocytes and erythrocytes are formed in the ectopic hemopoietic tissue; however, eosinophils and neutrophils are produced most abundantly.

9. Erythrocytes average 4.6 μ in diameter. Leucocytes in the venous vessels of the nodules comprise 17% of the cells present as contrasted to 0.3% in the arterial circulation, and are generally one-half to one-third the size of their counterparts in the general circulation of three other bat genera.

10. The possibility of experimental production of ectopic hemopoiesis in the bat's flight membranes and study of the relations of blood flow and vascularization to hemopoiesis in the living tissue is suggested.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Ventral view of left wing of *Pizonyx vivesi*, showing as blackened areas the approximate locations of all nodular areas observed in both wings of 23 specimens. 1, 2, 3 indicate the primary nodular areas; in area 1 nodules were present in 96% (44/46) of the wings, in area 2 in 63% (29/46), and in area 3 in 57% (26/46).

D.LA., dactylopatagium latus
D.LO., dactylopatagium longus
D.M., dactylopatagium minus
PL., plagiopatagium
PR., propatagium
UR., uropatagium (interfemoral membrane)

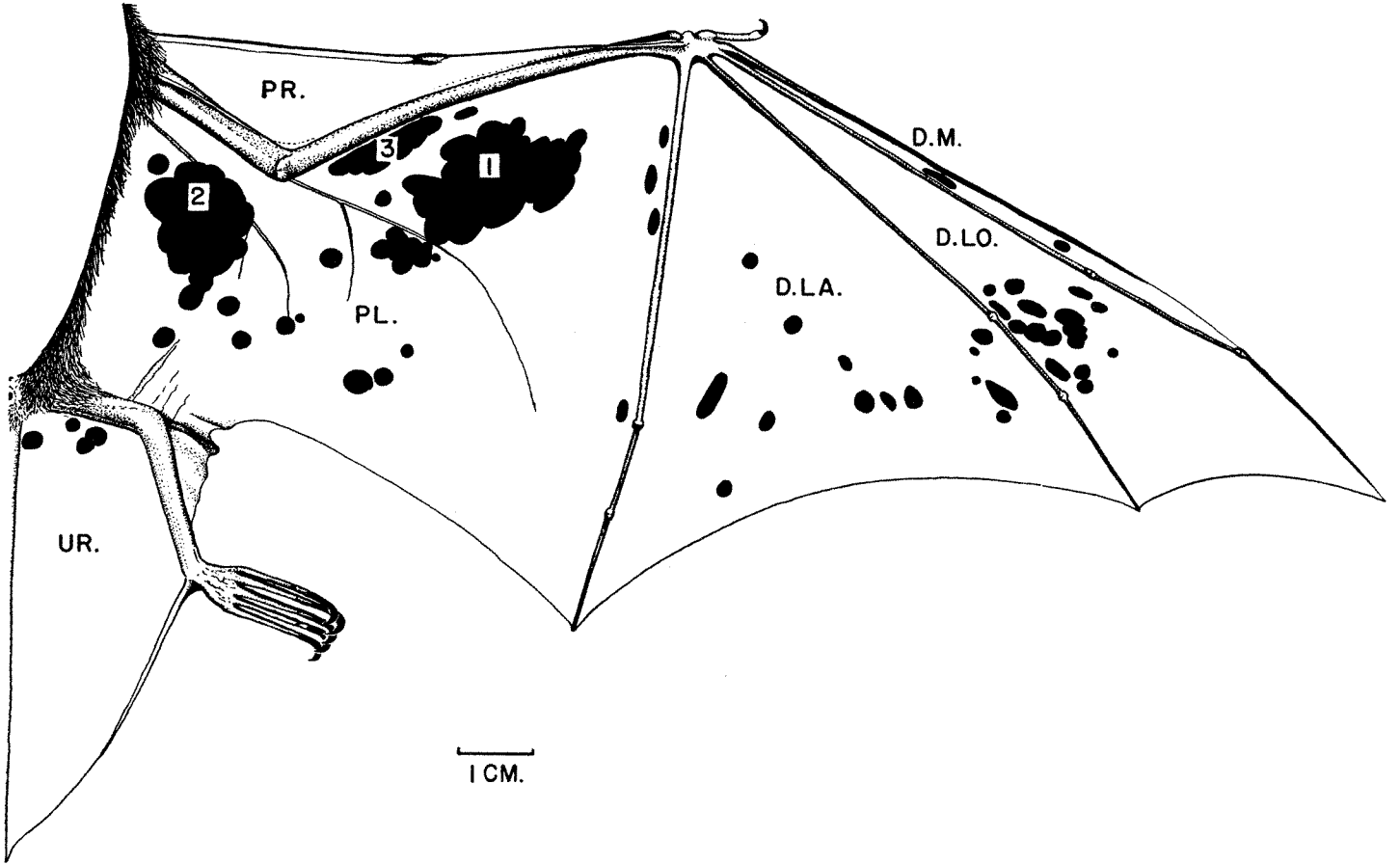
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PLATE 2

EXPLANATION OF FIGURE

Vertical longitudinal section of an area 2 nodule

The piece shown below is continuous with the one above. The right edge of the upper piece is continuous with the left edge of the lower one in the section. Phosphotungstic acid hematoxylin stain. Drawn with the aid of a camera lucida.

A., artery
AE., area of extravasated blood cells
E., elastic fiber bundles
EA., eroded area in epidermis
F., fibrin-like material
HF., hair follicle
SB., sebaceous gland
SK., skeletal muscle fibers
V., vein
VS., venous sinus

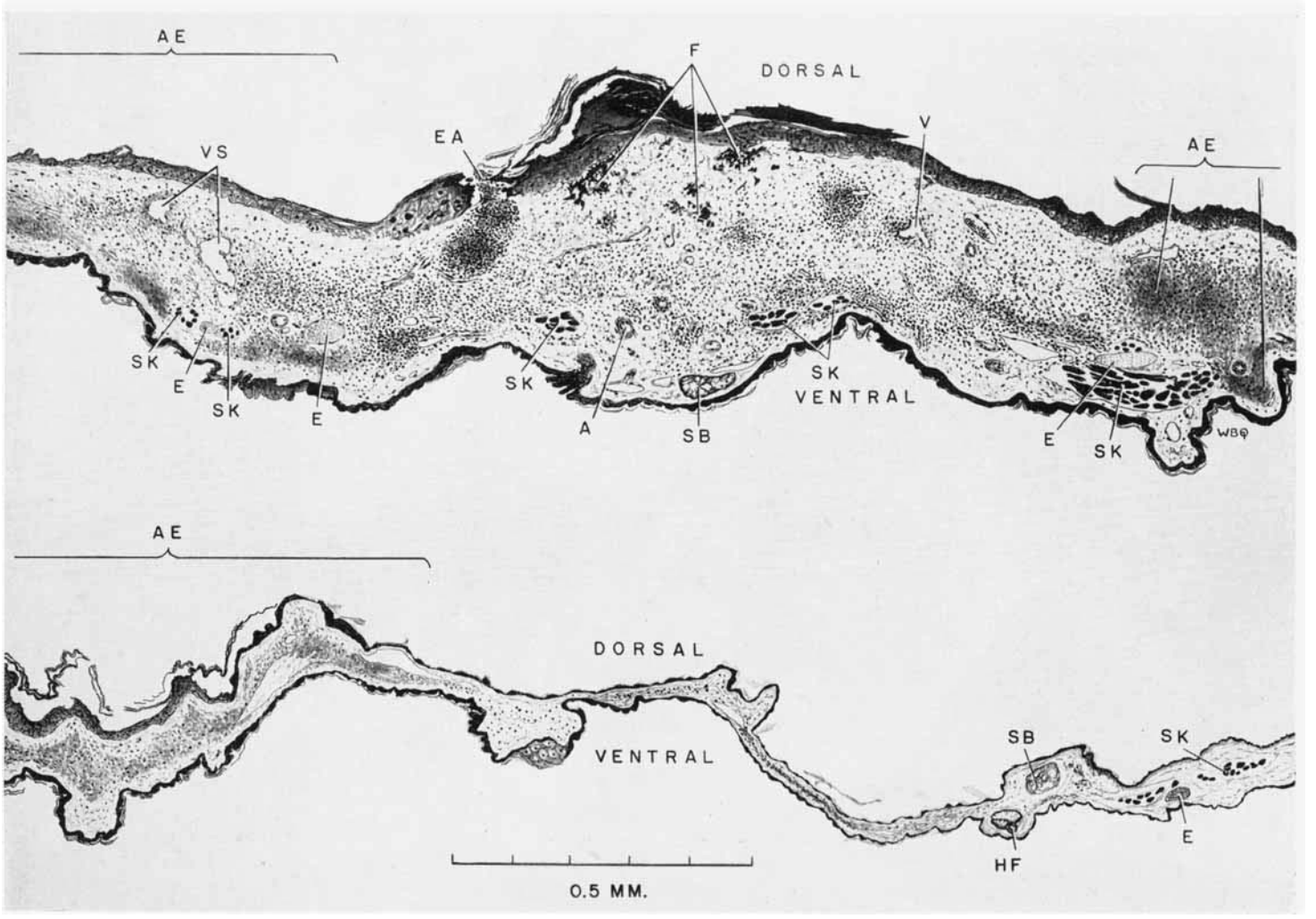
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PLATE 3

EXPLANATION OF FIGURE

Semidiagrammatic reconstruction of the vascular network and the epidermal derivatives of a small area within a wing nodule, viewed from the dorsum. The epidermal derivatives of only the upper surface of the wing are shown.

A., artery
CF., cystic follicle
EA., eroded area in epidermis
HF., hair follicle
LS., lymph sinus
SB., sebaceous gland
SD., sudoriferous gland
V., vein
VS., venous sinus

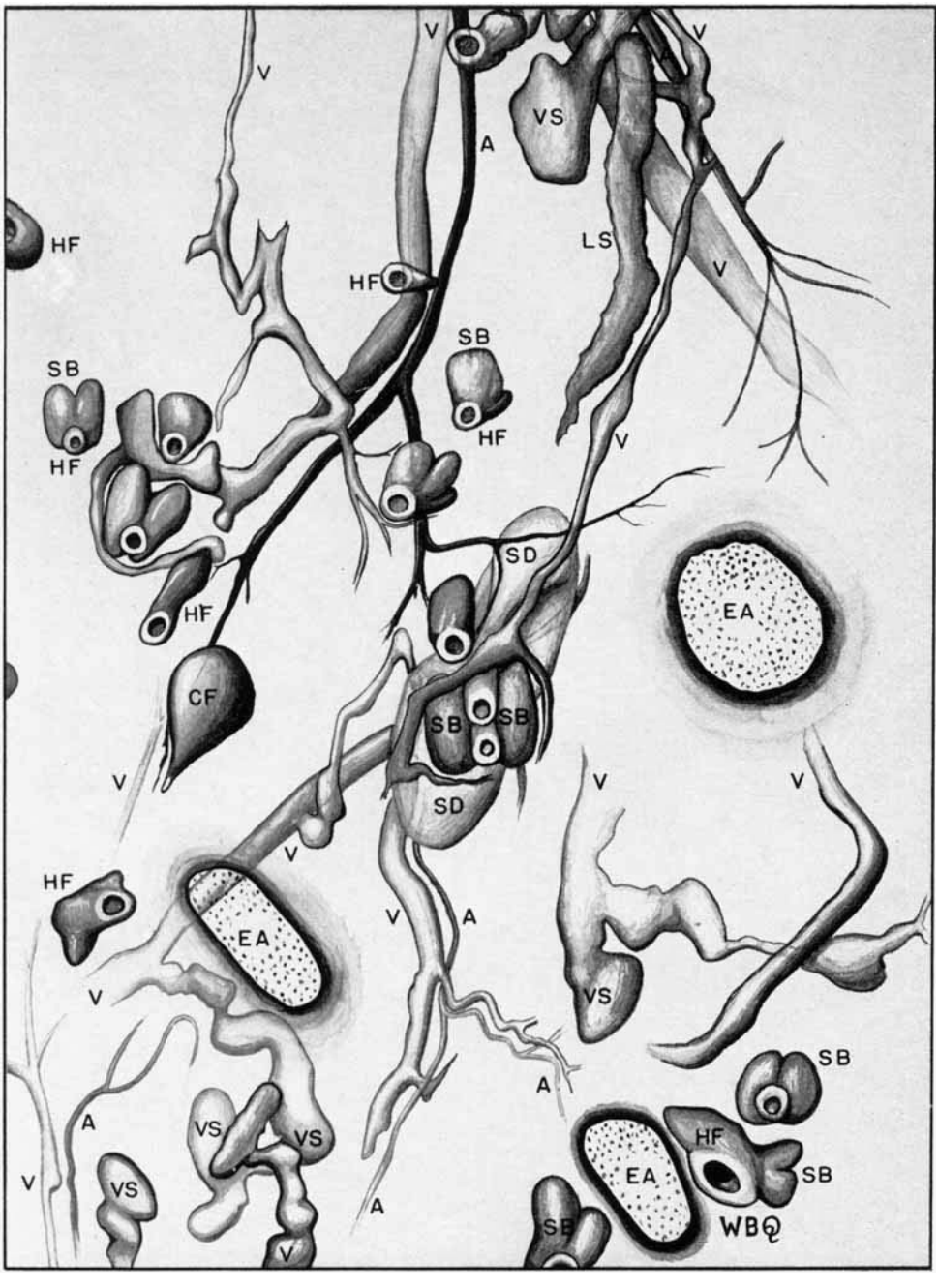
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PLATE 4

EXPLANATION OF FIGURES

4 Vertical section of an edge of an eroded area in the wing nodule epidermis. The edge of the epidermis, containing large epidermal cells with melanin granules, and two leucocytes, is at the upper right. The exposed surface of the leucocyte-filled connective tissue is at center left. Note particularly the abundance and locations of leucocytes and the formation of heavy collagenous fibers in the eroded area.

5 Reconstruction of elastic fiber bundles (black) and skeletal muscle fiber bundles (gray) in an area 2 nodule.

6 Aberrant cells of the erythroblast-normoblast series from the hemopoietic tissue of a nodule.

7 Typical area of nodule connective tissue, showing arterial (center right) and venous (lower left) ends of a capillary loop. Note the great variety of cell types in the delicate connective tissue surrounding the capillaries.

8 Small area of nodule connective tissue, showing cells most of which are in the myeloblast-neutrophil-granulocyte series.

9 Small area of nodule connective tissue, showing cells most of which are in the erythroblast-normoblast series.

All figures made with the aid of camera lucida and from hematoxylin azure-eosinate preparations.

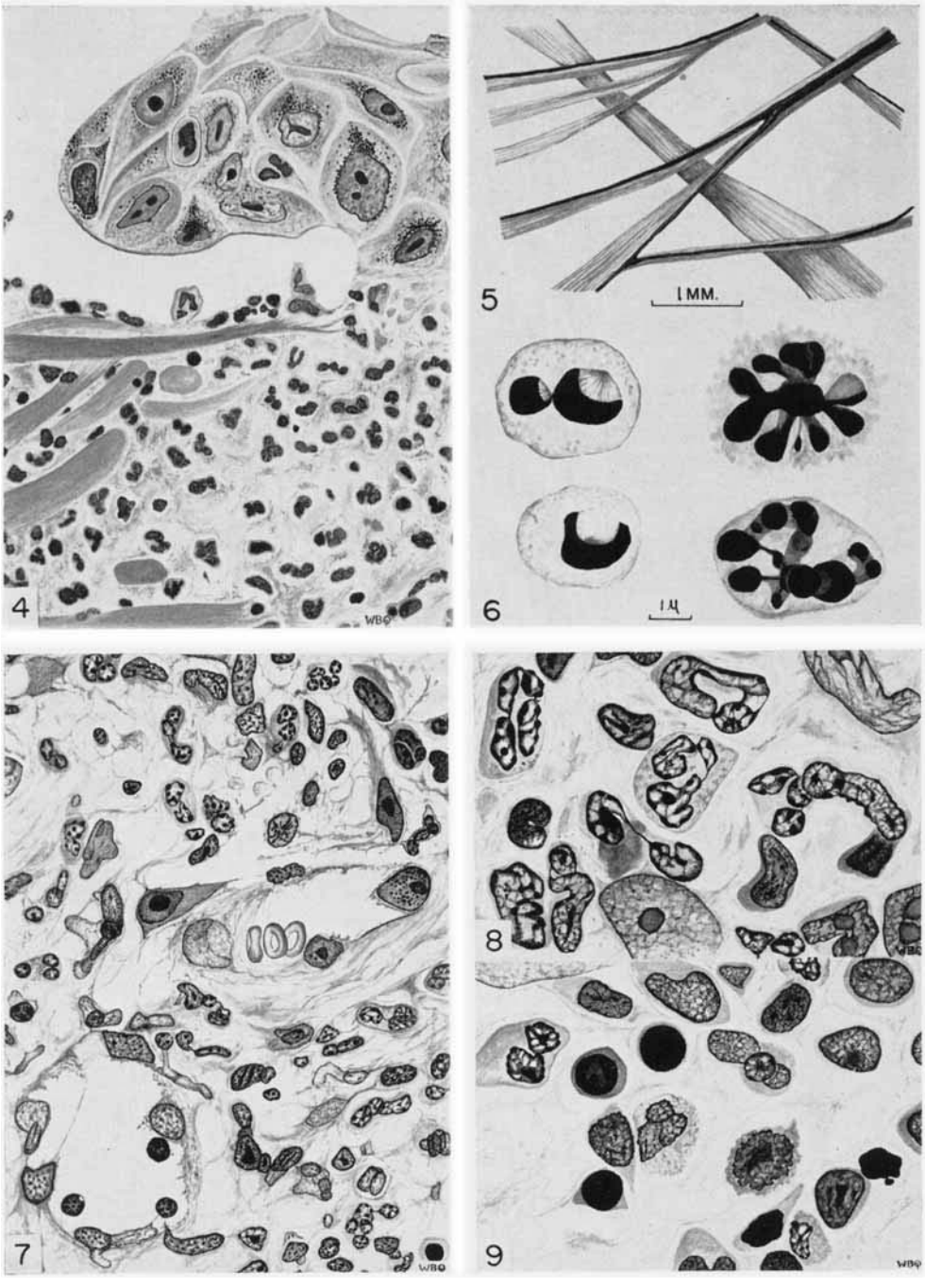
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PLATE 5

EXPLANATION OF FIGURES

Selected cells from an area 2 nodule. Hematoxylin azure-eosinate. × 5333.

10 Macrophage from an area of chronic extravasation. Note content of erythrocytes and brown granules.

11 Small late normoblast.

12 Small mast cell from nodule connective tissue.

13 Eosinophil.

14 Amoeboid wandering cell (lymphoid).

15 Polychromatophil erythroblast.

16 Typical erythrocyte.

17 Modified lymphocyte.

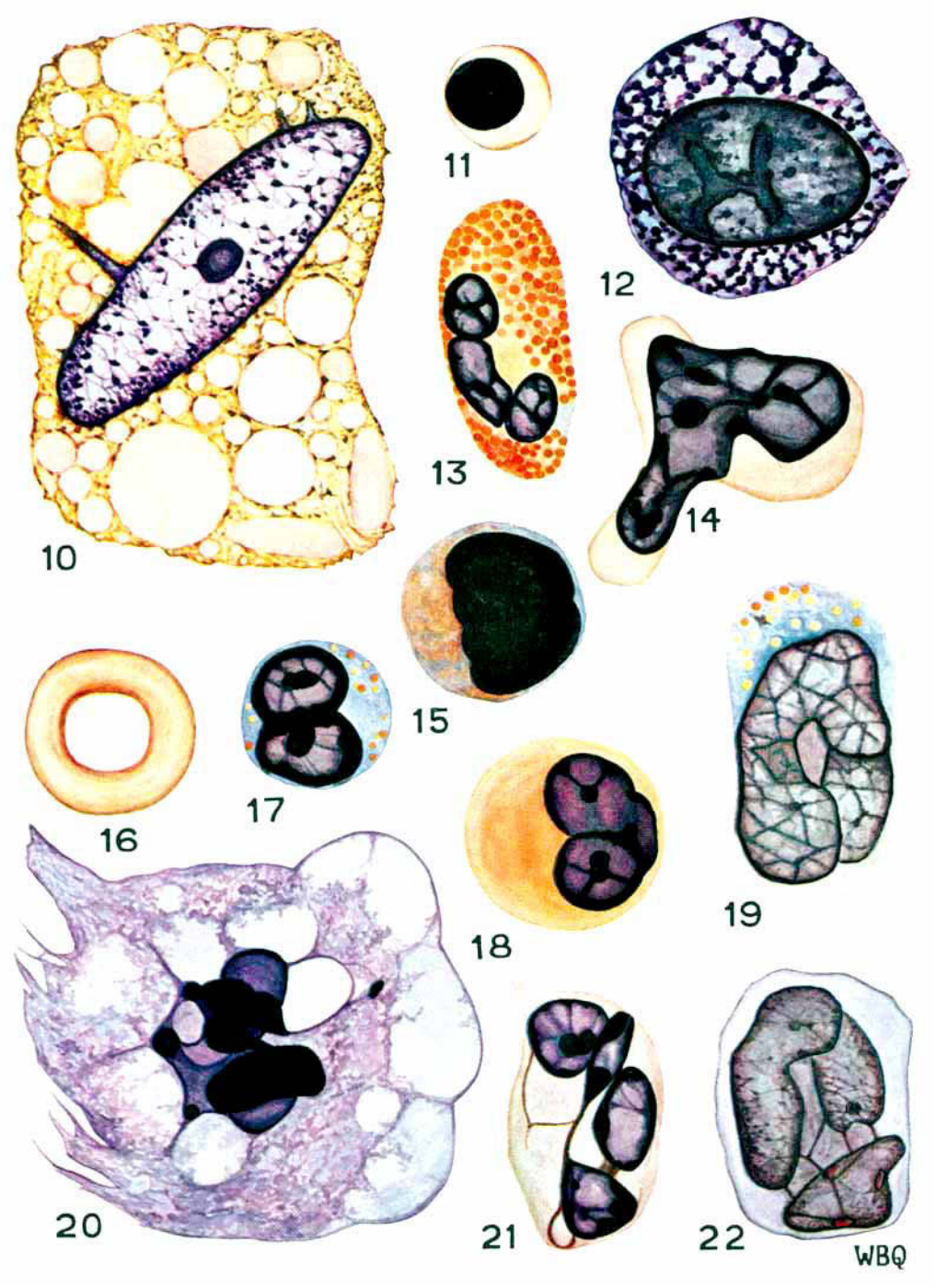
18 Early normoblast.

19 Eosinophil myelocyte.

20 Megakaryocyte.

21 Neutrophil.

22 Neutrophil myelocyte.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