A study on the cytomorphologic structure of blood cells by vital staining I. Normal human blood cells in the bone marrow

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Abstract

Vital observation on the cellular morphology of the normal human blood cells was conducted by means of bone marrow culture successfully in conjunction with vital staining with Janus green B and neutral red. A special attention was paid for the alterations of the cellular structures in the course of the culture. The findings are summarized as follows: 1) Intracellular particles with affinity to Janus green B or neutral red were classified into minute granules, granules, vacuoles, and mitochondria. Morphologic features of each type of the particles were studied in detail. 2) Two types of granules are present in neutrophilic and eosinophilic blood cells, whereas one type of granules is present in basophilic blood cells. Eosinophilic and basophilic granules show characteristic pole formation in them at the terminal stage of the staining. 3) The rosette formation in the mature monocyte and the aggregations of neutral red vacuoles in the mature neutrophil and the mature lymphocyte were characterized. 4) The cluster of neutral red vacuoles is characteristic of the erythroblast. 5) The mitochondria of the mature neutrophil and the mature monocyte participate in producing neutral red vacuoles.

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A STUDY ON THE CYTOMORPHOLOGIC STRUCTURE OF BLOOD CELLS BY VITAL STAINING

I. NORMAL HUMAN BLOOD CELLS IN THE BONE MARROW

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Indentification of each blood cell depends generally on the cytomorphologic structure specific to each cell series, but the deviation of the cellular structure in pathologic state frequently raises a trouble in cell identification. This is particularly true in the cases of leukemia. The development of modern cytology of the blood cells inaugurated by tri-acid staining of EHRlich and the further progress achieved by supravital staining introduced by Cowdry offered a great help in the identification of the cell series. However, there are still some difficulties to identify the morphologic characteristics specific to each cell series in the case where the cell structures are so severely deviated as in leukemia. To explore this problem, an attempt was made to clarify the cytomorphologic characteristics of blood cells in tissue culture combined with vital staining.

Although numerous cytologic studies on the blood cells by supravital staining or vital staining have been reported, combined staining with Janus green B and neutral red has not been applied to bone marrow culture except for the present investigation. This seems to be due to the fact that some dyes like Janus green B have high toxicity to cells and cause a quick death of the cells with consequent difficulty to observe the living cellular structure. It is the purpose of the present paper to present newly revealed morphologic characteristics of the various blood cells of the normal bone marrow in living state by means of bone marrow culture in conjunction with vital staining and to show the alterations of the morphologic structure in the course of the culture which seem to be specific to each cell series. In addition, this investigation serves as

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a basic study for the following paper in which cytologic deviations of leukemic cells will be presented in detail.

MATERIALS AND METHODS

A small fragment of the bone marrow tissue found in the aspirate from the sternum of normal individuals was used as an explant of the tissue culture. The simple method, which was devised in our laboratory was chosen as the tissue culture technic, since it proved convenient for vital observation on the cellular morphology of blood cells. Janus green B (the Matheson Co., U.S.A.) and neutral red (Merk) were added to the culture medium to give the final concentration of 1: 60,000 and 1: 10,000, respectively. The preparations were stored in an incubator regulated at 37°C and the observation was performed with the bright field microscope placed in a warm box at 37°C at the 1st, 3rd, 5th, 8th, and 12th hour of the culture and then every 6 to 12 hours for 72 hours. The vacuoles were distinguished from the granules by their peculiar characteristics. The vacuoles are spherical in shape, stained uniformly and enlarge in the course of the culture. The granules, on the other hand, are often stained irregularly and show no obvious enlargement throughout the entire period of the culture, though they appear spherical or oval in shape and may display slight swelling. The granules are approximately 0.3 to 1.0 μ in diameter, but the vacuoles frequently exceed this range. Exceedingly fine particles are defined as the minute granules which are stained a deep red with neutral red. Besides these three types of neutral red particles and the mitochondria stained with Janus green B, unstable vacuoles have been observed as refractile vacuoles which are spherical in shape and gradually grow large, showing no coloration through the entire period of the culture.

RESULTS

At the beginning of the culture, practically none of the cells shows stained particles. Approximately 10 minutes later, a few neutrophils scattered around the explant start to show the granules stained with neutral red. As time passes, neutrophils, eosinophils, basophils, monocytes and lymphocytes migrate out of the explant, forming a growth zone around it. Immature myeloid cells and erythroblasts, on the other hand, are seen staying near the explant without exhibiting an active migration and form the inner layer of the growth zone, since they have little motility. At about the 3rd to 5th hour of the culture, all of the cells other
than a small number of degenerated ones in the growth zone become stained in a maximum intensity with neutral red and/or with Janus green B. At the 10th to 15th hour, the cells which compose the outer layer of the growth zone and are kept stained fairly well until this period of the culture start decoloration and degeneration. These alterations proceed gradually from the outer layer to the middle layer and then to the inner layer, requiring 48 to 60 hours for the entire process. Similar changes occur in the explant itself: at approximately the 5th hour of the culture, the cells of the peripheral portion of the explant begin to take dyes and this coloration proceeds to the center of the explant in about 60 to 72 hours, followed by decoloration and cellular degeneration.

In the following account the morphologic changes of blood cells in the course of the culture will be described on the major cell series.

Neutrophilic Series

Promyelocyte: The promyelocyte is observed to be a large round cell and occasionally has needle-like processes or bleb formations on the cytoplasmic surface, but has no capacity of locomotion. The nucleus appears rather homogeneous, oval in shape and outlined with a very thin nuclear membrane. In the nucleus, there are one to three nucleoli which are obscure and somewhat yellowish in color. The cytoplasm is clear, colorless and moderate in amount. The mitochondria are of large size and round or rod in shape, staining greenish blue with Janus green B.

In the young promyelocyte, a large number of mitochondria are scattered along the shallow indentation of the nucleus, forming a loose aggregation in the cytoplasmic bay, and another small dense aggregation of the mitochondria is located next the nucleus in the portion of the cytoplasm most distant from the cytoplasmic bay (Fig. 1). In the central part of a mass of the mitochondria which is located in the cytoplasmic bay, a few minute granules are seen scattered near the Golgi zone. These minute granules which are deeply stained with neutral red, keep the color for several hours and frequently become tinged with a blue hue prior to decoloration. The minute granules do not show the tendency of vacuolization throughout the culture.

In the promyelocyte of the more mature stage, the mitochondria are observed to be distributed in the peripheral portion of the cytoplasm, surrounding the nucleus and red or yellow granules (Fig. 2). The mitochondria hold a greenish blue color for a few hours till subsequent decoloration. They do not show obvious morphologic changes during the culture. First in this level of the maturation, two types of granules stained red with neutral red can be observed, i.e. A type and B type. The granules of A
type appear spherical or oval in shape, measuring approximately 0.3 μ in diameter and stain a light yellowish red owing to their poor affinity to neutral red, whereas those of B type, which are less in number, stain a deep red with neutral red. These two types of granules are very similar in size and shape except for the difference in affinity to neutral red. They, mingling with each other, occupy the central portion of the cytoplasm and are encompassed by a circular arrangement of the mitochondria, forming a small to large sphere in proportion to the maturation of the promyelocytes. These granules are kept stained for 10 to 15 hours and fade without any change in size.

The myeloblast, namely a cell in which there are no neutral red particles seen despite the existence of a large number of mitochondria, is hardly encountered in the normal bone marrow.

Myelocyte: The myelocyte is a cell which is most frequently encountered within the explant (Fig. 4). It has no specific features different from the promyelocyte except for the lack of nucleoli and of stained mitochondria.

Metamyelocyte and Neutrophil: The metamyelocyte and the neutrophil migrate actively in the growth zone, pushing pseudopods in and out from the head of the cell (Fig. 5). The nucleus appears kidney-shaped, rod-shaped or lobulated. The granules have a far less affinity to neutral red than those of the immature neutrophilic cells, although the two types of granules are recognized. The cytoplasm except for the pseudopod is filled with the granules. In the base of the pseudopod, several mitochondria are readily observed moving quickly in Brownian movement. These mitochondria are, unlike those of the promyelocyte, small in size, rod in shape and stain a faint bluish violet. With a precise observation, many mitochondria of the same type are seen in the whole cytoplasm to shift with the granules in the cytoplasmic flow, quivering with Brownian movement. Many of the mitochondria are kept stained for a few hours. Neutral red vacuoles are seen near the Golgi zone as early as 30 minutes from the start of the culture. At first, they are very small and are stained faintly with neutral red. As time passes, they are, increasing in size, number and color intensity, scattered into the whole cytoplasm except for the pseudopod. The red coloration of the vacuoles reaches the maximum intensity 5 to 6 hours later. At this time, the cells may appear to be filled with many large neutral red vacuoles. The coloration of vacuoles gradually decreases in intensity and disappears in about 8 to 12 hours from the onset of the staining. This is the major process of forming neutral red vacuoles, but another mechanism is ob-
served. At the terminal period of the culture, some of the mitochondria become swollen and tinged with a reddish hue. Subsequently a small colorless vacuole develops on the surface of these discolored mitochondria. This vacuole gradually increases in size and stains with neutral red. The neutral red vacuoles of this type do not seem to reach such a large size as another type does.

**Eosinophilic Series**

The eosinophilic promyelocyte has many red granules in the central portion of the cytoplasm and many dark greenish blue mitochondria encircling the large aggregation of the granules and the nucleus (Fig. 3). In the youngest eosinophilic promyelocyte, the granules appear round in shape, and slightly larger than those of the neutrophilic promyelocyte, staining deep red with neutral red. In the more mature eosinophilic promyelocyte, two types of granules are recognized, i.e. A type and B type. The granules of A type appear oval or lozenge in shape, measuring approximately 0.5 to 1.0 μ in diameter and stain light yellow or orange yellow with neutral red. A red pole develops occasionally at one end of the granule at the terminal stage of the coloration. The granules of B type appear round in shape, measuring about 0.3 μ in diameter and stain rather deep red with neutral red. The coloration of the granules fades in about 10 hours. The mitochondria of the eosinophilic promyelocyte are similar to those of the neutrophilic promyelocyte in size, shape, coloration and change in the course of the culture. In the eosinophilic myelocyte, the eosinophilic metamyelocyte and the mature eosinophil, the two types of granules described above fill the whole cytoplasm, whereas the mitochondria are rarely seen (Fig. 9). Neutral red vacuoles are likewise scarcely seen in eosinophilic blood cells. The other cytologic features of the eosinophilic series are similar to those of the neutrophilic series.

**Basophilic Series**

The immature basophilic blood cell has round violet red granules of medium size sparsely in the cytoplasm and mitochondria of large size among the granules. In the mature basophil (Fig. 10), the mitochondria lose the affinity to the dye. In the later stage of the culture, the granules undergo swelling and a violet red pole develops in each end of the granule. The coloration of the granules disappears in 10 to 15 hours after the onset of the staining. The other cytologic features are not very much different from those of the other types of the granulocytes except for the round and indented nucleus.

**Monocyte**

The monocyte is observed to be a large thin cell, having a good
motility with membraneous pseudopods projected from the entire cytoplasmic margin (Fig. 7). The nucleus is usually lobulated. At first, several minute granules stain a deep red near the Golgi zone and as time passes, the minute granules become quickly enlarged and form neutral red vacuoles. These neutral red vacuoles are arranged in a typical rosette pattern which was first described by Sabin. The numerous mitochondria which surround the rosette and the nucleus are small rod in shape, and stain blue. After the mitochondria have lost coloration in several hours, the whole cytoplasm appears to be occupied by many neutral red vacuoles which often reach more than 1.0 \( \mu \) in diameter. Some of the mitochondria participate in the formation of neutral red vacuoles by the same mechanism as seen in the neutrophils. The coloration of neutral red vacuole disappears in about 10 to 15 hours.

**Lymphocyte**

The lymphocyte is observed to be a small round cell which occasionally has needle-like processes (Fig. 8). Forming a pseudopod, the lymphocyte sometimes migrates in the culture medium. The nucleus is round or kidney-shaped. Several neutral red particles which are first recognized as minute granules develop into neutral red vacuoles. They are distributed around the nucleus, but occasionally form a small aggregation in the cytoplasmic bay. The several mitochondria are likewise scattered around the nucleus or in the cytoplasmic bay. They are of large rod shape like those of immature granulocytes and stain a dark blue. Neutral red vacuoles and mitochondria are kept stained for several hours.

**Erythroblast**

The erythroblast is observed to be a round mononuclear cell, varying in diameter from that of an erythrocyte to that of a promyelocyte (Fig. 6). The cytoplasm is usually outlined clearly from the surrounding medium and appears thick at the margin, possessing neither processes nor motility. The color of the cytoplasm varies from light yellow to yellow according to the hemoglobin content. The nucleus is round in shape and clearly outlined, including a small nucleolus or nucleoli in the erythroblast of the early developmental stage. At the beginning of the staining, several minute granules are observed to be located in a circular fashion around the Golgi zone with a small number of minute granules scattered in the cytoplasm. As time elapses, these minute granules gradually increase in diameter and turn into neutral red vacuoles. Thus a cluster of neutral red vacuoles is produced around the Golgi zone. This cluster appears very much like a rosette or more exactly a rose nosegay. This double or triple circle of neutral red vacuoles is considered to be
characteristic of all the erythroblasts. In a rosette of the monocyte, on the other hand, neutral red vacuoles are more diffusely arranged in a radial fashion. The cluster of neutral red vacuoles does not grow large enough to occupy the entire cytoplasm prior to its decoloration. The mitochondria are large in size, rod in shape and stain a greenish blue with Janus green B. In the young erythroblast which is the counterpart of the young promyelocyte, the mitochondria are distributed in a semisphere of the cytoplasm among which the cluster exists. In the erythroblast of the late developmental stage, the mitochondria are scattered around the nucleus, accompanied by a small aggregation of mitochondria encircling the cluster of neutral red vacuoles. The mitochondria usually disappear before the erythroblast gives rise to an erythrocyte. In a small number of the erythrocytes, however, a few mitochondria stained with Janus green B are seen in the cytoplasm along with neutral red vacuoles.

DISCUSSION

There are many publications concerning supravital observation on the blood cells, but the terms a “granule” and a “vacuole” are very often confused, both meaning nothing but a particle stained with neutral red. A few publications describe the definition of the granule and vacuole. According to Sabin, granules are particles which are stained in a uniform manner and which remain stationary in size after exposure to a dye such as neutral red. Vacuoles, on the other hand, are stained fluid particles which are capable of undergoing variations in size and color, provided a chemical indicator such as neutral red is employed. Morita explains a vacuole as a mixture of neutral red and viscous fluid accumulated around a pre-existing granule, and a neutral red granule, on the other hand, as a pre-existing granule itself which is stained with neutral red. The author agrees fundamentally with Sabin's definitions, but owing to difficulty in recognition of change in size in a short period of observation, other features of vacuoles have been added to her definitions as described before in this paper. Since the basic concept of a vacuole is considered to be a fluid particle in the cytoplasm, a vacuole should be spherical in shape because of surface tension of the fluid, and stained uniformly, because vacuoles are thought to be composed of practically homogeneous fluid. On the contrary, since granules are considered to be solid particles, it may be reasonable that granules keep their size and shape stationary during culture, unless their dissolution takes place and that a granule could stain in a mosaic fashion, since a granule may consist of parts varying in the
composing substances. The dissolution of the granules was not observed in the present study. According to the author's experience, the definitions of the present paper seem to be quite reasonable in describing the fine cellular morphology of the blood cells.

**Neutrophilic Series:** On the myelocyte A which corresponds to the young promyelocyte of this paper, SABIN et al.\textsuperscript{12}, using the supravital technic, have described that the first step in this transformation from myeloblasts to myelocytes is the development in the cytoplasm of a small clump of neutrophilic granules or their precursors. Other investigators\textsuperscript{11} have also described similar findings. In the present study, in addition to this finding, it has been demonstrated as essential features of these granules that the granules, or minute granules of the present paper, actually do not increase in size in the course of the culture, and often become tinged with a blue hue at the late stage of the culture. These minute granules, furthermore, are scattered in the cytoplasmic bay much more loosely than those of the erythroblast which invariably surround the Golgi zone in a circular fashion. As the cells following myelocyte A, SABIN defines myelocyte B and myelocyte C by the number of the neutrophilic granules and she simply states that the granules of these cells stain more intensely in neutral red than the neutrophilic granules of the adult leukocytes. The differentiation of A type and B type granules, however, takes place in the stage of these types of myelocytes which correspond to the promyelocytes of the present report, as claimed by ACKERMAN and BELLIOS\textsuperscript{1}. As for the granules of the myelocyte and the mature neutrophil, the result of the present study is fairly consistent with Ackerman's description\textsuperscript{1}, but no specific tendencies have been observed concerning the distribution of the A type and the B type granules. The neutrophilic granules C of Ackerman's paper should be described as neutral red vacuoles. The present observation on the mitochondria of the promyelocyte is compatible with that described by many investigators such as SABIN et al.\textsuperscript{12}, SIMPSON and DEMING\textsuperscript{13}, MORITA\textsuperscript{14} etc. There has been an argument on the presence of mitochondria in neutrophils, however. SIMPSON et al\textsuperscript{13} claim that in the transformation of myelocytes into leukocytes there is usually an entire loss of the mitochondria from the cytoplasm. CUNNINGHAM and TOMPKINS\textsuperscript{4} describe that mitochondria are few or lacking in the neutrophils. MORITA\textsuperscript{14} states that mitochondria disappear at the level of the myelocyte. UETANI\textsuperscript{16} has observed a few mitochondria in the neutrophils. In the present study, however, many mitochondria are observed invariably to exist in the cytoplasm and they are usually stained a faint violet blue. This observation is consistent with that of ACKERMAN and BELLIOS\textsuperscript{1}, although they claim that in
neutrophils there are a few mitochondria which fail to stain with Janus green. The presence of mitochondria in the neutrophils has been demonstrated by phase contrast microscopy in our previous study\textsuperscript{8} and by WATANABE\textsuperscript{17} with the electron microscope. A small number of mitochondria of mature neutrophils have, interestingly enough, a capacity of producing neutral red vacuoles as described previously in this paper.

**Eosinophilic Series:** SIMPSON and DEMING\textsuperscript{13}, using the supravital staining, state that the process of development of the eosinophilic granules seems identical in neutrophils and eosinophils and that the granules assume the characteristics peculiar to these cell types when they begin to coarsen and become refractive. As previously described in this paper, however, the granules of the most immature cell recognizable as an eosinophilic promyelocyte are rather numerous and intermediate in size between neutrophilic granules and mature eosinophilic granules. The eosinophilic promyelocyte which has a small number of mature eosinophilic granules like that of rabbits illustrated in MORITA's paper\textsuperscript{14}, is not encountered in the normal human bone marrow. These findings lead the author to presume that the eosinophilic promyelocyte of the highest level where a small number of eosinophilic granules or their precursors are postulated to exist is morphologically identical with the young neutrophilic promyelocyte in which a few minute granules are seen near the nucleus. The mitochondria are rarely seen in mature eosinophils, as claimed in the papers of CUNNINGHAM and TOMPKINS\textsuperscript{4}, and UETANI\textsuperscript{16}. The two types of granules are distinguished in mature eosinophils in the present study in opposition to the statements of CUNNINGHAM and TOMPKINS\textsuperscript{4}, and ACKERMAN and BELLIOS\textsuperscript{1}. This finding confirmed TAMURA's description\textsuperscript{15}.

**Basophilic Series:** SIMPSON\textsuperscript{13} states by the supravital technic that the development of the basophils appears a similar plan to that of the other granulocytes, but there are very few specific granules in the young basophilic myelocytes, so that a zone of specific granules about the centriolar region is not so conspicuous as in the other granulocytes. She also claims that the specific granules of the basophil begin to scatter when there are still very few of them present. The present observation has confirmed her description. However, the basophilic promyelocyte of the same level as the young neutrophilic promyelocyte seems to be morphologically similar to the latter. The presence of mitochondria of the mature basophil has not been demonstrated in the present study. They are, however, considered to be present with no affinity to Janus green B\textsuperscript{8}. It is interesting that the basophilic granules produce a violet pole in each end of them at the terminal stage.
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of the coloration.

**Monocyte:** Although a specific feature of the monocyte has been claimed by Sabin\(^3\) to be a rosette arrangement of neutral red vacuoles, the rosette has been observed in many different types of cells such as lymphocytes, plasma cells, reticular cells, etc\(^6\). In the present study, however, it has been found that the rosette of the monocyte is a loose aggregation of a large number of neutral red vacuoles stained equally salmon-red and arranging in a radial fashion about the Golgi zone with larger vacuoles at the periphery. On the contrary, in the rosette of neutrophils, if present, the neutral red vacuoles vary in color: some are almost colorless and others are stained red. The lymphocyte often shows rosette formation, but it is far smaller in size than that of the monocyte. As time passes, the neutral red vacuoles of the rosette of the monocyte become larger and larger, and finally occupy the entire cytoplasm. At this stage of the culture, the mitochondria are not observed in the monocyte.

**Lymphocyte:** Despite SABIN's definition of granules and vacuoles, she\(^3\) often used the term "granules" for describing the neutral red particles of the lymphocyte as well as of the monocyte. At the beginning of the culture, the lymphocyte and the monocyte show fine neutral red particles, i.e. minute granules in the cytoplasm. As time passes, these minute granules obviously increase in size, thus forming neutral red vacuoles. SABIN's descriptive confusion is considered due to the fact that since cells undergo a rapid degeneration in supravital preparations, one is not allowed to observe the cells long enough to enable minute granules to form vacuoles. There has been a question whether the neutral red particles, or minute granules of the monocyte and the lymphocyte are small neutral red vacuoles or solid bodies. It is the author's opinion that these minute granules are solid, and possibly correspond to small granules demonstrated by the electron microscope\(^10,17\) and that these minute granules change into neutral red vacuoles presumably owing to their high solubility to water.

**Erythroblast:** SABIN\(^5\) does not illustrate an erythroblast containing neutral red vacuoles and AMANO\(^2\) states that there are neutral red vacuoles in some of erythroblasts. In the present study, however, the presence of minute granules stained a deep red has been found to be an invariable finding of the erythroblast as claimed by ACKERMAN and BELLIOS\(^1\). It has furthermore been demonstrated that in the course of the culture, these minute granules develop into neutral red vacuoles which form a cluster about the Golgi zone. This cluster formation is considered to be characteristic of the erythroblasts of all the developmental stages, although the neutral red
vacuoles of the plasma cell may occasionally produce a cluster of this type.

SUMMARY

Vital observation on the cellular morphology of the normal human blood cells was conducted by means of bone marrow culture successfully in conjunction with vital staining with Janus green B and neutral red. A special attention was paid for the alterations of the cellular structures in the course of the culture.

The findings are summarized as follows:

1) Intracellular particles with affinity to Janus green B or neutral red were classified into minute granules, granules, vacuoles, and mitochondria. Morphologic features of each type of the particles were studied in detail.

2) Two types of granules are present in neutrophilic and eosinophilic blood cells, whereas one type of granules is present in basophilic blood cells. Eosinophilic and basophilic granules show characteristic pole formation in them at the terminal stage of the staining.

3) The rosette formation in the mature monocyte and the aggregations of neutral red vacuoles in the mature neutrophil and the mature lymphocyte were characterized.

4) The cluster of neutral red vacuoles is characteristic of the erythroblast.

5) The mitochondria of the mature neutrophil and the mature monocyte participate in producing neutral red vacuoles.

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REFERENCES

3. CUNNINGHAM, R. S., SABIN, F. R. and DOAN, C. A.: The development of leucocytes, lymphocytes and monocytes from a specific stem-cell in adult tissue. Contributions to Embryology, No. 84, 227, 1925
5. DOAN, C. A., CUNNINGHAM, R. S. and SABIN, F. R.: Experimental studies on the origin and maturation of avian and mammalian red blood cells. Contributions to Embryology. No. 83, 163, 1925
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11. Sabin, F. R., Doan, C. A. and Cunningham, R. S.: The discrimination of two types of phagocytic cells in the connective tissue by the supravital technique. Contributions to Embryology, No. 82, 125, 1925

EXPLANATION OF ILLUSTRATIONS

1. Young neutrophilic promyelocyte. Minute granules are encompassed by a number of large rod-shaped mitochondria in the wide portion of the cytoplasm with several mitochondria in the opposite side. 3rd hour of culture.
2. Neutrophilic promyelocyte. An aggregation of neutrophilic granules is surrounded by large rod-shaped mitochondria. 3rd hour.
3. Eosinophilic promyelocyte. Eosinophilic granules are surrounded by large rod-shaped mitochondria. 3rd hour.
4. Neutrophilic myelocyte. Neutrophilic granules fill the cytoplasm. Mitochondria are not seen. 3rd hour.
5. Mature neutrophil. Neutrophilic granules and faintly stained mitochondria fill the cytoplasm. Several neutral red vacuoles are seen. 5th hour.
6. Erythroblast. A cluster of neutral red vacuoles are encompassed by several large rod-shaped mitochondria. 10th hour.
7. Mature monocyte with a rosette and fine mitochondria. 6th hour.
8. Mature lymphocyte. Neutral red vacuoles and large rod-shaped mitochondria are scattered around the nucleus. 10th hour.
10. Mature basophil. Basophilic granules fill the cytoplasm. 6th hour.
Schematic Illustrations of Normal Human Blood Cells in the Bone Marrow.

1. Minute granules
2. Neutral red granules
3. Mitochondria
4. Neutral red vacuoles or Nucleolus

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