An Electron Microscope Study of Sectioned Cells of Peripheral Blood and Bone Marrow

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This is a report of a study dealing with the fine structure of human blood cells, especially leukocytes, based on thin sections examined with the electron microscope. It was hoped that the high resolving power of the electron microscope might reveal, in sectioned blood cells, structures not previously recognized, and that a survey of normal material would later provide a basis for studies of submicroscopic pathology of blood cells in various hematologic disorders. Extensive use in this laboratory of the phase contrast microscope for the study of both living and fixed blood and bone marrow cells has provided us with abundant material for reference and comparison.

So far as we can ascertain, this is the first attempt to section leukocytes for electron microscopy. Considerable experimentation on methods and techniques of preparation of material has therefore been necessary. None of the procedures tried has proved to be wholly satisfactory; results have often been capricious, and much further work is necessary before reliable and constant results are to be expected. Yet the methods reported here show some promise of providing a useful approach to hematology, and hence our experience to date is reported in preliminary form in order to stimulate interest in this important field of endeavor.

Literature

A few reports in the literature deal with the submicroscopic anatomy of red blood cells as revealed by electron microscopy. The isolated red cell membrane or "ghost" was among the earliest of animal structures to which electron microscopy techniques were applied. Biochemical analyses and polarization microscope studies of the plasma membrane have been very fruitful. Physicochemical and biochemical studies, and especially x-ray crystallography, have yielded a limited amount of information, and have led to a great deal of conjecture, concerning the nature of the "cytoplasm" of the mature erythrocyte.

As regards the ultrastructure of white blood cells, we have found no reports in the literature dealing with sectioned material. Some cytoplasmic details have been visualized by Bessis and Bricka, Bernhard et al., and Porter et al., using thin-spread areas of leukocytes grown in vitro on collodion films. Even such thin areas are, however, often too thick to be penetrated by the electron beam, and granules and other formed elements appear only as opaque bodies, with little hint as to their internal structure. Several other workers have studied whole leukocytes which were air dried and shadowed, ruptured, digested, or microincinerated. All of these methods of preparation are more or less traumatic and are designed to bring out certain elements of the cell at the expense of others. Other electron and light microscope studies have been concerned with various isolated components of blood cells, such as nuclei and nuclear fragments, mitochondria, specific granules, and other cytoplasmic inclusions. These studies have the drawback of providing limited information about in vivo orientation and structure of such bodies as have been removed or selectively stained for study.

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MATERIALS AND METHODS

Ten to 20 cc. of human venous blood were drawn into a heparinized syringe, mixed thoroughly to inhibit clotting, and transferred to Kahn tubes for centrifuging. Blood was spun at 2000 or 2500 r.p.m. for 5 minutes, and the buffy coat thus obtained was pipeted off into fixative. In some cases a modified Kahn tube, drawn to a narrow neck in the middle, was used in order to facilitate separation of the buffy coat layer. After removal of the plasma, the tube could be broken just below the buffy coat layer, and the cells readily blown out into fixative. Due to mechanical injury of the cells in early cases, it seemed advisable to enclose the cells in some material which would hold them together and permit easy handling. This was accomplished by mixing the buffy coat with gelatin warmed just to the liquid point (10 Gm. gelatin in 100 cc. normal saline), allowing this to gel in the refrigerator, and cutting out small pieces of about 1 cu. mm. volume for fixation. It was found advantageous to place the blood in fixative as soon as possible after removal from the patient.

In our work the buffy coat was in fixative in less than ten minutes after it was drawn.

Although the initial objective of this research was a study of the cells of normal peripheral blood, technical difficulties made it necessary to use other material initially. Peripheral blood of patients with high white counts, and often counts high in a single cell type, were used to increase the yield of leukocytes. Marrow from the femur of young rabbits was utilized to establish certain conditions of fixation and embedding. Both normal and abnormal human bone marrow aspirations were also used. In the latter, marrow was removed by sternal puncture and injected directly into fixative. In these cases normal clumping was sufficient to keep the cells together and prevent excessive injury due to handling.

Fixatives used were buffered 10 per cent formalin and osmium tetroxide (osmic acid). Only osmic acid provided satisfactory fixation and sufficient hardening for ultra-thin sectioning. The OsO₄ was buffered with Michaelis' veronal-acetate buffer. A pH of between 7.25 and 7.35 was found to be most satisfactory. Concentrations of OsO₄ ranging from 0.1 per cent to 1.0 per cent were tried. Of the various dilutions used, 0.25 per cent fixative appeared to be the most satisfactory. In order to prevent possible rupture of the cells, an attempt was made to correct the tonicity of the fixative to a more nearly physiologic level, usually by adding 0.4 Gm. KCl per 100 cc. fixative.

Tissue was fixed for from two to four hours and dehydrated in ethanol, usually without intermediate rinsing in water. A relatively rapid dehydration, as recommended by Palade, seemed to produce better results than did passing the tissue slowly through a closely graded series of alcohols. The blocks were then embedded in N-butyl methacrylate monomer, which was subsequently allowed to polymerize under the influence of ultra-violet light.

It should be emphasized that the methods reported here did not always produce satisfactory results. Indeed, fixation was found to vary even within a single block of tissue. Apparently some unknown variables in our technic still stand in the way of uniformly reproducible results. Concentration of the fixative, pH, tonicity, length of time of fixation, as well as the washing and dehydrating schedule, all seem to be important in this respect.

A number of methods reported in the literature as suitable for other tissues were found to be entirely unsatisfactory in our work on peripheral blood and bone marrow.

The hardened blocks were trimmed to about 1/2 mm. on a side, and were sectioned with glass knives on a modified Spencer rotary microtome. The sections were floated out on 50 per cent acetone in distilled water. Sections of suitable thinness (0.05µ or less) were selected and mounted on formvar-coated electron microscope grids. These were studied in an RCA model EMU electron microscope, equipped with a biased gun.

Electron micrographs were taken at original magnifications of 4000 to 6000 X. Further photographic enlargement was employed as indicated. Polysterene latex particles, with an assumed diameter of 2500 A, were photographed for calibration purposes.

OBSERVATIONS

Most cell types normally found in peripheral blood, and some members of the erythroid and myeloid series usually found only in bone marrow, have been tentatively identified. It must be remembered that our present knowledge of
the anatomy of white blood cells is based almost entirely on the study of whole, and usually somewhat flattened cells. Also, tissue “stained” with osmic acid presents a picture of dark and light structures dependent on differences in electron scattering power and affinity for a heavy metal. On the other hand structures which stand out when viewed with the light microscope do so because of differences in light refraction, or in affinity for various dyes. Optical sections from a given block of tissue were frequently studied with the light microscope for the purpose of identifying predominant cell types, but neighboring ultra-thin sections were still often difficult to evaluate.

Since it is virtually impossible to obtain a pure sample of any single blood cell type rapidly enough to permit the necessary immediate fixation, we were obliged to study a great many mixed cell samples from the blood of a number of different patients before the basic cell types could be identified with any degree of assurance. Only those cells which were sectioned through the nucleus, and appeared to be free from mechanical injury and reasonably well fixed, could be profitably studied. Strands of fibrin, tangential sections of cells, the gelatin used for embedding, and considerable cellular debris could often be identified in the surrounding material. Only those cells which we feel show the best fixation so far obtained are described below. Preservation of known intracellular detail, lack of evidence of shrinkage, fineness and homogeneity of the protein materials precipitated by the fixative, and appearance of the same structural details in cells fixed under varying conditions were taken as some of the criteria for “good” fixation. The submicroscopic anatomy of several cell types is described below.

**Neutrophils (Figures 1 and 2)**

This cell type may be recognized by the typical shape of its nucleus and the numerous inclusions which undoubtedly represent the neutrophilic granules. The nucleus appears relatively homogeneous and rather finely precipitated by the fixative. With satisfactory fixation the nuclear membrane appears clearly visible and quite thin. The cytoplasm appears somewhat more finely granular than the nucleus, and its electron scattering power is slightly less. It contains occasional small vacuoles, possibly representing areas where lipid inclusions were located in the living cell, or they may represent areas where granules have fallen out of the section. The numerous granules appear homogeneous and very dense. They have a clearly defined limiting membrane. Their greatest diameter in sectioned material is about \( \frac{1}{4} \mu \), and the smallest less than \( \frac{1}{10} \mu \). The smallest sized structures appear in such numbers as to suggest that they may possibly represent a second species of granules or mitochondria rather than tangential cuts of the larger ones. Mitochondria of the type seen in other leukocytes (described below) have not been clearly identified in neutrophils in our studies, although occasional inclusions display indistinct internal structures.

**Monocytes (Figure 2)**

Our identification of monocytes in this material receives confirmatory support from the fact that the buffy coat from a patient known to have a high white count especially rich in monocytes showed these cells most abundantly in electron microscope studies. The sectioned cell is, in general, only a little smaller
Fig. 1.*—Polymorphonuclear neutrophil. The numerous granules (g) are seen to advantage. The small sectioned granules (sg) may represent a second species of organelle. Indistinct internal structure suggests that several of the larger bodies may be mitochondria (m?). Strands of fibrin surround the cell. The nucleus is sectioned approximately through its center and shows the typical horse shoe shape. Cell from the bulky coat of a patient with 30,000 white count. × 13,500.

than the largest sectioned neutrophils. Its nucleus has an electron density similar to that of the neutrophil. In some of our micrographs the double nature of the nuclear membrane is evident. The nucleus shows typical hofs or indentations in several instances. Under the conditions of fixation employed, the cytoplasmic material has precipitated in a rather sparse network. Numerous vacuoles of

* All electron micrographs shown are of sectioned human material, fixed in buffered osmic acid as noted in the text. Identification of the various cell types is based on parallel studies of optical sections of the same material, studies of bulky coat from patients with white counts high in a single cell type, and on obvious structural details which resemble those of stained and unstained cells viewed with the light microscope. Identifications are, nevertheless, tentative. Sections are estimated to be 0.05μ or less in thickness. Approximate final magnifications are given in each case.
Fig. 2.—Monocyte (upper left) and part of a neutrophil (below). The nuclear membrane of the monocyte is seen to be double (indicated by arrow). The structureless vacuoles (v) may represent osmophobic lipids which have been dissolved out during dehydration. The complex internal structure of the mitochondria (m) may be seen in the insert, which shows a portion of another monocyte. The numerous striped mitochondria (described in text) are collected near the hof of the nucleus. Cells from the buffy coat of a patient with a high monocyte count. Insert from the blood of a chronic myelogenous leukemia patient. × 13,500, insert × 15,000.

Varying sizes are seen in some cells. Some of these spaces appear empty and possibly represent dissolved out lipid inclusions of the osmophobic type. Others have varying degrees of internal structure and are interpreted as the mitochondria found in monocytes (fig. 2, insert). These mitochondria show a well defined limiting membrane and, in longitudinal section, exhibit pairs of filamentous structures running approximately perpendicular to the long axis of the mitochondrion. This often gives the mitochondria a striped appearance. In some cross sections these structures are arranged in cartwheel fashion, being closely applied to the limiting membrane of the mitochondrion and fading out near its center. Similar internal structure of mitochondria has been noted by
Weinstein\textsuperscript{51} in chicken cardiac muscle and Sjöstrand\textsuperscript{52} and Palade\textsuperscript{53} in various other tissues. Several other workers in this laboratory have also noted occasional striped mitochondria in other tissues. Whether this is a common feature of all mitochondria, to be seen only when they are exceptionally well fixed and thinly sectioned, is not known.

\textit{Lymphocytes (Figure 3)}

Several patients with chronic lymphocytic leukemia were used as a source of blood rich in lymphocytes. Cells corresponding in relative size and shape to lymphocytes were found abundantly in this material and also occasionally in material from other individuals. Both cytoplasm and nucleus of the osmic acid fixed lymphocyte possess much greater electron scattering power than any of the other cell types of peripheral blood. Much of the nuclear material is clumped in a coarse network and is found especially along the nuclear membrane. The background material of the nucleus is less dense than the clumped material but

\textbf{Fig. 3.—Lymphocytes. The dense nuclear material is coarsely precipitated. Several striped mitochondria are seen in the cytoplasm. A longitudinal section of a filamentous mitochondrion is also present (arrow). Sectioned from the buffy coat of a chronic lymphatic leukemia patient.} $\times$ 15,000.
is still darker than the nuclei of most other cells. The nucleus shows a typical hof in many of our micrographs. The lymphocyte cytoplasm is also dense, and rather finely precipitated. The mitochondria are seen to be elongated rods, somewhat longer in relation to their width than are the mitochondria of monocytes. They also often exhibit the striped appearance described above. The mitochondria are not numerous in most lymphocytes and show a tendency to collect near the hof. From phase microscope studies it is known that lymphocytes contain two species of mitochondria: the majority are of the long filamentous type while a second variety is small and round. The distinction between these two types has not yet been clearly established in our studies because of the small numbers of mitochondria found in each cell and the limited probability that a filamentous structure will be sectioned longitudinally. The majority of mitochondrial sections we have seen in lymphocytes have been approximately round, and most of these probably represent cross sections of long filamentous mitochondria. No details have been noted which would distinguish these from sections of the round mitochondria.

Eosinophils (Figure 4)

The numerous very large, highly osmophilic granules of the eosinophil make it easy to identify even when only fragments of cells are seen. The nucleus shows the typical lobed or horse shoe shape and is of medium density with the fixation employed. There is some tendency for denser nuclear material to aggregate along the nuclear membrane, although this condition is not nearly as pronounced as that seen in sectioned lymphocytes. Several of our micrographs (e.g. fig. 4a) show a denser central spot which probably represents the nucleolus. The eosinophilic granules are of extreme interest. They appear to have two components: a very dense central substance possessing extremely high electron scattering power, hence appearing black in the micrographs, and a lighter matrix or surrounding portion. It is not clear whether a limiting membrane is present; some granules show an indistinct border and in others no membrane can be distinguished. The inner portion appears, in some granules, to have a short rectangular or barrel shape. In some other views the dark portion of the granule seems to be quite round, about \( \frac{1}{2} \mu \) in diameter, and seldom of even density throughout. In these, little or no gray matrix surrounds the dark central core. In still other granules the dark substance takes the form of a cartwheel or flower with three to six lobe-shaped petals. The lighter substance in these granules is seen to fill the remainder of a rounded area about \( \frac{1}{2} \mu \) in diameter. Dark rods about \( \frac{1}{2} \mu \) long and \( \frac{1}{15} \) to \( \frac{1}{4} \mu \) wide are also seen, sometimes surrounded by a narrow margin of the lighter substance. If the dark core of the granule be viewed as a somewhat barrel-shaped cylinder, deeply grooved in three to six places along the sides (to produce the lobes seen in end views), the narrower rods might represent longitudinal sections of single lobes—these would appear when a granule was cut tangentially—or they could represent fragments of ruptured granules. Elsewhere in the cytoplasm a few elongate mitochondria of the striped variety may sometimes be seen. Mitochondria, as distinguished from acidophilic granules, have not been seen in the cytoplasm of eosinophils in the phase microscope studies carried out in this laboratory. In 1914, Cowdry\(^{54}\) demonstrated
Eosinophils (Figure 4).—Eosinophils. The cell in 4b shows a bilobed nucleus. A dense nucleolus (nu) is seen within the nucleus in 4a. The specific granules (described in text) are seen to possess a dark inner core which is sometimes round (gc), sometimes made up of several lobes (gel), sometimes barrel shaped (gel), and sometimes in the form of narrow rods (gcr). A lighter gray matrix (ma) surrounds the core in most cases. Some of the granules appear to have a limiting membrane (lm). A few striped mitochondria (m) are also seen. 

(a) From normal marrow; (b) from the buffy coat of a patient with a 30 per cent eosinophilia. × 15,000.

small numbers of mitochondria in eosinophilic myelocytes by staining with Janus green, but their presence in mature eosinophils is not often appreciated.

Basophils (Figure 5)

Because of the scarcity of basophils and their apparent fragility under the present conditions of fixation and embedding, very few examples have been found. A portion of a cell from a normal marrow aspiration exhibits two large, grayish, rather homogeneous bodies which we believe to be basophilic granules. The nucleus of this cell is cut tangentially, but displays an elongate, somewhat indented form. The egg-shaped granules are almost 1 μ long and show a distinct
Fig. 5.—Portion of a cell presumed to be a basophil. The nucleus is indented, and the cytoplasm sparsely precipitated. Several large granules (g) are seen, and also some smaller dark spherical objects occurring in aggregates (ag) (see text). Mitochondria (m) and vacuoles (v) also appear in the cytoplasm. Cell obtained from normal marrow. X 15,000.

limiting membrane. They have been seen in several other instances in which the cell to which they belong has apparently been only partly included in the section (fig. 6a). Often associated with them are groups of much smaller, darker, spherical objects which resemble aggregations of droplets of varying sizes. They are gathered together like droplets of coalescing oily or fatty substance, and their pronounced osmophilia suggests that they could indeed be lipoid in nature. The significance of these aggregations is not known. Striped mitochondria are also found within the cytoplasm in several cases.

Other cells

A number of cells have been found in the marrow preparations which have not yet been identified, but which nevertheless show interesting cytoplasmic detail. Extensive fibrillar structures, usually paired and arranged in concentric rings about the nucleus, have been seen occasionally and are shown to advantage
Fig. 6.—(a) Cell believed to be a member of the erythroid series. The nuclear material is clumped. The cytoplasm appears homogeneous except for some fibrillar material (fib) to the right of the nucleus. To the right of the erythroblast are several large granules (bg), presumably from a basophil. Small dark droplets (ag in fig. 5) are seen nearby. Taken from normal marrow. X 17,500.

(b) An unidentified marrow cell exhibiting paired cytoplasmic fibrils or membranes (f). Mitochondria (m) are seen between the filamentous structures in several places. The dense, rather homogeneous nucleus contains a nucleolus (nu). Cell from normal marrow. X 15,000.

in figure 6b. In some cases these cells also contain large elongate mitochondria encircled by the fibrillar structures. An occasional fibrillar or tubular structure is seen randomly oriented in the cytoplasm of other cells, reminiscent of the "endoplasmic reticulum" of Porter and Palade and the paired cytoplasmic membranes of Sjöstrand. Only a few cells have been studied in which this fibrillar network seems to comprise the entire cytoplasm. Bernhard et al. have described "chains of corpuscles" and "granular filamentous structures" in the cytoplasm of leukemic cells and have postulated as to their virus nature. The fibrillar structures described in our material have been seen in both normal and abnormal marrow, although it should be noted that in all cases they appear in
cells which are probably young. It is also of interest that Birbeck found similar structures in some cells of the rat spleen fixed with OsO₄ and studied in the electron microscope: “the cytoplasm of the spleen contains a large number of parallel lines as though there are multiple membranes present.” Rinehart and Farquhar describe similar structures in rat anterior pituitary cells as “cytoplasmic canaliculi or lamellae”.

Several cells have been tentatively identified as members of the erythroid series (fig. 6a). Their cytoplasm is usually homogeneous, although some fibrillar material is visible in the cytoplasm of the cell shown. We know from phase microscope studies that erythroid cells have filamentous mitochondria, but we have not yet seen enough sectioned erythroblasts to demonstrate these. The nuclear material is clumped in a rather coarse network along the nuclear membrane as well as throughout the middle of the nucleus.

A great many erythrocytes have, of course, been seen in our sectioned material. Satisfactory fixation conditions for red blood cells have been found to differ markedly from those currently employed for leukocytes. Since our efforts have been directed toward the best obtainable fixation of leukocytes, we have little to report on well fixed red cells. Indeed, as might be expected, little can be seen in the sectioned red cell that has not already been studied extensively by other means. The plasma membrane can be clearly seen, and is of the order of 200 to 300 Å thick. No internal detail has been observed, although the hemoglobin molecule is of a size well above the limits of resolution of the electron microscope and should be detected if the red blood cells are well fixed. Most of our micrographs show longitudinal ridges and cracks in the erythrocytes, an artifact which we believe may be related to the differences in consistency of OsO₄-fixed red blood cells, other cells, and the embedding plastic, and the sectioning difficulties incumbent on this condition.

**DISCUSSION**

Studies on whole leukocytes by other workers have demonstrated specific granules and or mitochondria in the cytoplasm, and occasionally some granular or fibrillar details of submicroscopic dimensions. Since blood cells are normally too thick for penetration by the electron beam, these studies have necessarily depended on altering cells by air drying, spreading on a film in vitro, rupturing, digestion, autolysis, or microincineration, with the increased possibilities of artifact production accompanying such techniques. Phase contrast studies, because they can be done on living cells, are actually more revealing up to the limits of resolution of the light microscope system.

Ultra-thin sectioning techniques, not previously applied to white blood cells, permit one to obtain material which can be penetrated by the electron beam, without subjecting the cells to harsh and often destructive preparatory procedures. Osmic acid appears to be much more satisfactory as a fixative than any other conventional fixatives used either in our studies or those of others. We do not present the methods reported as necessarily ideal, or even consistently reproducible, nor is this study regarded as exhaustive. We feel, however, that artifacts are kept at a minimum by the procedures used, and that our best micrographs represent reasonably authentic pictures of white blood cells.

The methods employed have enabled us to identify many of the various blood
cell types with some degree of assurance. We have observed almost all structures within the various cell types that one would expect to find from knowledge previously obtained by ordinary microscope and phase contrast studies.

On the submicroscopic level, the fibrillary nature of certain marrow cells, as well as occasional random fibrils in other cells, is described. It is significant that in our work the cytoplasm of leukocytes from peripheral blood, either normal or leukemic, does not show extensive or oriented fibrillation, in contrast to the reports of Bessis and Bricka on normal leukocytes and of Bernhard et al.23-34 on leukemic cells. Both of these reports seem to indicate that the cytoplasm of these cells is essentially filamentous or fibrillar. Attention is called to the studies by Porter, Claude, and Fullam55 on electron microscopy of cultured cells, in which the authors illustrate alterations which can result from fixation. That the fibrillar network often described in leukocytes is at least in part a fixation artifact is also the conclusion of Haguenau and Bernhard22 who were able to demonstrate varying degrees of network formation with different fixatives. Porter and Kallman46 have recently reached similar conclusions in a study of OsO4-fixation of pith blocks impregnated with various proteins.

Observations on the specific granules of granular leukocytes indicate that neutrophilic and probably basophilic granules have a distinct limiting membrane and a rather homogeneous interior. On the other hand it is not clear whether eosinophilic granules have a limiting membrane, but their internal structure is quite complex, and varies from one granule to another.

We have in addition observed the detailed structure of mitochondria: they are seen to have a limiting membrane and inner lamellae, giving them a striped appearance, similar to that of mitochondria in other cells as described by Palade53 and by Sjöstrand.52 The presence of mitochondria in granular leukocytes, established by Cowdry54 with vital stains, is not often appreciated because of the masking effect of the numerous granules.

**Summary**

White blood cells from peripheral blood and bone marrow have been sectioned for study in the electron microscope. Methods of fixation and handling are described. Most of the usual blood cell types have been tentatively identified, and their fine structure is described. The high resolving power of the electron microscope promises to reveal details previously unsuspected, as well as to extend and clarify existing knowledge concerning the cytology of blood cells, both normal and pathologic.

Ultra-thin sectioning, while still a very difficult art, appears to be the best method currently available for visualizing the fine structure of white blood cells, which would otherwise be too thick for penetration by the electron beam. Conditions of satisfactory fixation and dehydration are extremely critical, and care must be exercised in the interpretation of all results in order to separate gross fixation artifacts from the finer precipitation of protoplasmic material which may approximate a true picture of the living cell.

**Summario in Interlingua**

Leucocytas ab sanguine peripheric e ab medulla ossee ha essite sectionate pro studios a microscopio electronic. Detalios technic, includite le fixation, es descri-
STUDY OF SECTIONED CELLS

The majority of the usual type of blood cells has been tentatively identified and their fine structure is described. The higher power of the electron microscope promises to reveal details previously suspected, and it will extend and clarify the existing knowledge concerning the cytolgia of blood cells, normal and pathologic.

The sectioning ultra-thin, although still extremely difficult, is the better method currently available for visualizing the fine structure of the leukocytes that is not visible in thin sections of the electron microscope. This will reveal details not previously suspected and extend and clarify existing knowledge concerning the cytology of blood cells, normal and pathologic.

The sectioning of blood cells is still extremely difficult, but the better method currently available for visualizing the fine structure of the leukocytes that is not visible in thin sections of the electron microscope promises to reveal details not previously suspected and extend and clarify existing knowledge concerning the cytology of blood cells, normal and pathologic.

REFERENCES

KAUTZ AND DEMARSH


STUDY OF SECTIONED CELLS

An Electron Microscope Study of Sectioned Cells of Peripheral Blood and Bone Marrow

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