The Phagocytosis of Leucocytes*

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The surface tension theory seems to have been often referred to in explaining the mechanism of phagocytosis.^{7),12),37)} According to this theory, the devouring of a foreign body can be performed without biophysical and biochemical energy. Activities of respirations and glycolysis have been measured as to phagocytizing cells. The results of these measurements, however, do not seem to have totally denied the surface tension theory.

The present study originated from a study on relations between phagocytosis and energy metabolism in an attempt to clarify the mechanism of phagocytosis.

I. Relationship between Energy Metabolism and Phagocytosis of Leucocytes

We already reported that the movement of leucocytes originated in a mechano-chemical system which is coupled by ATP.^{13-18),41-44),46)} Then, we have made an approach from energy metabolism to relations between phagocytosis and motile function. The present experiment was performed in vitro with India ink phagocytosis.

When the blood is added with ATP, there develops an acceleration in the motile function and phagocytosis of leucocytes. But the addition of arsenate and phloridzin which are inhibitors of ATP results in a decrease in both motile and phagocytic function. The addition of arsenate plus ATP or the addition of phloridzin plus ATP results in more acceleration of both functions than control, although it shows slightly fewer functions than ATP alone. It is revealed that the inhibitory action of arsenate and phloridzin against both functions is counteracted by ATP. And the addition of ATP to the ascites of the guinea-pig results in a greater acceleration of India ink phagocytosis of peritoneal macrophages than control, as in neutrophils. Thus ATP has been found to be the entity of the energy of phagocytosis as disclosed in case of movements of the

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cell.^{9), 10), 43)}

A considerable number of reports has been made of the system of glycolysis and respiration from which leucocytes derive energy.^{2-5), 8), 22), 24), 25), 30-35)} In the present experiments this system is studied in reference to specific features of leucocytes. As a result, it is revealed that the glycolysis of neutrophils in motion is higher than that of those at rest and that aerobic glycolysis is lower than anaerobic glycolysis in both moving and resting neutrophils, i.e., Pasteur effect is evidenced.³⁶⁾ Movement is maintained longer under aerobic conditions than under relatively anaerobic conditions. India ink phagocytosis shows no such difference between these two conditions. The movement and phagocytosis of neutrophils show no significant differences for a certain period under these two conditions. However, as the production of energy decreases with the lapse of time, the movement of cells gradually decreases, while phagocytosis is well maintained.

Hence, evidence of dissociation between phagocytosis and movement of cells.⁹⁾

II. Dissociation between Phagocytosis and Movement of Leucocytes

The aforesaid dissociation observed between phagocytosis and the movement of leucocytes indicates that these two functions have common factors as well as uncommon factors. Therefore, it seems that the pursuance of the cause of the dissociation affords a useful clue to the clarification of the mechanism of phagocytosis. The first approach was made to the mode of the activities of

Fig. 1. Five motile types of neutrophils. Each type shows 4 stages of periodic movement which are repeated. Of these types, type I is observed only in metamyelocytes of the marrow, the others in mature neutrophils of the blood.

individual leucocytes, the dynamic pattern of neutrophils previously described by the present authors seem feasible^(13-18), 39-44), namely, neutrophils of healthy human adults are classified into five dynamic patterns (Fig. 1) which are each possessed with almost definite chemotactic and galvanotactic functions. A decrease in the motile function of leucocytes is accompanied by a corresponding shift in their dynamic patterns (Fig. 2). When the production of ATP is inhibited,

Fig. 2. Serial changes of movement of neutrophils. Refer to the present text for detailed information.

type II rapidly shifts to type III and IV, whereas an addition of ATP results in a retrograde shift from type III to type II, as shown in the broken line in the Figure 2. Type IV is what has developed an irreversible degeneration which can not be returned to type III. Thus, the motile form and function of leucocytes are co-ordinately controlled by ATP, and each dynamic pattern individually represents the activities of neutrophils. Subsequently relations between the shift of dynamic patterns and phagocytic function were comparatively pursued with the lapse of time. As a result it was disclosed that there obviously developed a dissociation between the motile and phagocytic functions in about 12 hours after the beginning of experiment. In correspondence with this period, types III and IV which show a decreased motile function become predominant in number. Addition of arsenate to the blood resulted in an earlier appearance of the above dissociation than a control. In this case, types III and IV were likewise predominantly observed at a more initial stage.⁽¹⁰⁾

The above findings lead us to interpret that the dissociation between India ink phagocytosis and the movement of leucocytes developed when types III and IV were predominant. This interpretation is applicable to the fact that even types III and VI which show decreased motile function due to decreased energy are able to phagocytize India ink particles.

The dissociation phenomenon is most conspicuously demonstrated by the erythrophagocytosis of neutrophils. There are two types in the phagocytosis of neutrophils on sensitized erythrocytes, i.e., whole erythrophagocytosis which devours a whole erythrocyte, and bisect erythrophagocytosis which cuts off part of an erythrocyte and devours it. The former is mostly found in types III and IV which show a decreased motile function, but seldom found in types II which shows

an active motile function. The development of the dissociation in whole erythrophagocytosis is paradoxical. On the other hand, the latter is only found in type II.¹⁰⁾

There is also found a dissociation between the pinocytosis and motile function of leucocytes, although it is not so marked as in India ink phagocytosis. The pinocytic function of neutrophils (in bovine serum albumin and globulin solution as well as in human serum γ globulin solution) is most active in type IIC whose motile function is slightly decreased.³⁸⁾

It is revealed that neutrophils show different phagocytic activities depending upon the kind of foreign body to be devoured.

III. The Mechanism of Phagocytosis

An attempt was made to clarify various conditions which are responsible for performing phagocytosis with ease or difficulty as mentioned above. Thus, the process of phagocytosis has been pursued with a phase contrast and electron microscopic approach.

a) Whole Erythrophagocytosis¹⁰⁾: When studied with a phase contrast microscope, type IIA which shows an active function is seen to approach an erythrocyte with the pseudopod and then pushes the erythrocyte forward by the advancing pseudopod. As a result, the intensive advancing power of the pseudopod separates the erythrocyte which adhered to the surface of a slide glass. Hence, the so-called surface phagocytosis occurs only with some difficulty. Furthermore, the pseudopod occasionally proceeds in a deviated direction after it touches the erythrocyte, and the cell is kept apart from the erythrocyte. Such phagocytic failure seems to be attributable to a high surface tension of the pseudopod. Types III and IV which show a decreased function adhere to the erythrocyte with their thinner pseudopod. The pseudopod slowly begins to enclose the erythrocyte. Then the granuloplasm enters the pseudopod. This process is progressively repeated until the erythrocyte is completely enclosed. In this case the erythrocyte keeps almost the initial position. The above findings lead to a disagreement with reports which are supported by electron microscopic findings only, that a foreign body is trapped into the cytoplasm of cells by indentation, or invagination of the cell membrane.^{6), 20), 23)}

It has been clarified that phagocytosis first requires the development of the pseudopod, and that it is greatly related with the adhesiveness and surface tension of the pseudopod. Thus, it seems adequate and necessary to discuss the above attributes of the pseudopod.

The pseudopod plays an influential part in phagocytosis, and an even more influential part in the cell movement so that the development of the pseudopod can even represent the cell movement. This seems to indicate that the pseudopod is closely related with the fact that some features are common and others are

uncommon between phagocytosis and the cell movement.

Suffice it to say, because of the limited space that only an outline conclusion about the development of the pseudopod is given here. From our experimental results it is assumed that contractile protein which seems to be of an actomyosine nature may exist in the surface layer of the granuloplasm of leucocytes, and that protein contracts by means of ATP. The contraction is observed morphologically as a wave, i.e., the constriction wave consists of the elongating and the shortening wave (Fig. 3). The former shows a circular contraction, pushing the

Fig. 3. Dynamic pattern of the elongating wave (vertical density) and shortening wave (horizontal density) of neutrophil.

cell body forward, and the latter shows a longitudinal contraction, drawing the cell body forward. The above contraction results in a formation of the pseudopod out of a fluid substance in the cytoplasm, which is squeezed out.^{44), 46)} We call the fluid substance an easily liquidifying substance. Accordingly the activity of the pseudopod represents the motile function of cells.

Next, it is obvious that the lower the surface tension of the pseudopod, the easier the phagocytosis. Since there is no way of directly measuring the surface tension of the pseudopod, we have tried to assume the extent of surface tension on the basis of morphological features of the pseudopod. When neutrophils take types III and IV with decreased activities, the pseudopod becomes broader and thinner in its contents and less tonic in its margin, showing a general membranous appearance. If viewed laterally, the cell body looks flatter. When non-ionic surface active agents are added to the blood, the engulfing activity of bacteria is increased. The pseudopod at this stage is membranous and feeble, being similar to that of type III and IV. From the above findings, it is assumed that the surface tension is the highest in type IIA and decreased in types III and IV which show a decreased production of energy.^{45), 46)}

Regarding the examination of the adhesiveness of the pseudopod, we have contrived a device by which measurement is performed under a direct microscopic visualization. Thus, it is revealed that type IIA possesses the highest adhesiveness followed by types III and IV in descending order.¹⁹⁾

Coming back to whole erythrophagocytosis, type IIA shows a high

adhesiveness but because of its high surface tension and the high developing power of the pseudopod, it fails to show a surface phagocytosis since it separates erythrocytes which adhered to the surface of a slide glass. But types III and IV, although they are low in adhesiveness, possess a low surface tension. It still leaves enough power to enclose an erythrocyte so that it is capable of functioning as a whole erythrophagocytosis.

Since phagocytosis is a dynamic phenomenon, it is advisable to fix an object being phagocytosed *in situ* for observation. Attention has been given to this aspect in preparing sections used in the present electron microscopic experiment. First electron microscopic observations were made of moving cells. In this case, the pseudopod of cells such as various leucocytes^{26), 27)} and peritoneal macrophages^{28), 29)} revealed no smooth surfaced endoplasmic reticulum or outward opening. Although it has been reported that openings of the smooth surfaced endoplasmic reticulum are observed in the pseudopod,^{48), 49)} our results have denied such findings, confirming that the smooth surfaced endoplasmic reticulum exists only in the granuloplasm. But it is to be noted that peritoneal macrophages occasionally cause pinocytosis so that the presence of a pinocytosis droplet in the pseudopod gives an appearance as if there were a smooth surfaced endoplasmic reticulum.

When relations between the growth of a neutrophil from a myeloblast and the endoplasmic reticulum is observed, it is revealed that the endoplasmic reticulum is abundant in a non-phagocytic promyelocyte but scanty in an active phagocytic neutrophil.²⁷⁾ Therefore, it led us to consider that the endoplasmic reticulum has nothing to do with phagocytosis.

On electron microscopic findings whole erythrophagocytosis²⁶⁾ is, like on phase contrast microscopic findings, started off with the contact, and the enclosing of an erythrocyte by the pseudopod through transference of the erythrocyte into the cytoplasm. On the outside of the phagocytized erythrocyte there is observed a limiting membrane which is connected with the cell membrane of the pseudopod (Photo. 1). This connection is subsequently separated. Accordingly it is obvious that the erythrocyte is devoured being enclosed by the cell membrane of the pseudopod and that this cell membrane is not easily digested in the cytoplasm of the leucocyte.

b) Bisect Erythrophagocytosis¹⁰⁾: Bisect erythrophagocytosis, if viewed by a phase contrast microscope, is carried out first by trapping a part of the erythrocyte with the pseudopod of the leucocyte and after granuloplasm having reached this part a constriction wave develops. This constriction wave moves along the margin of the trapped erythrocyte. The elongating wave makes the erythrocyte squeeze into a prolonged shape. The shortening wave draws the trapped erythrocyte inward. Especially the terminal, the part where the erythrocyte is trapped, shows an intensive contraction, resulting in a bisection of

the erythrocyte (Fig. 4). It seems reasonable that this bisection is carried out only by type II which shows the most active function.

Fig. 4. The process of bisect erythrophagocytosis. The arrows show the constriction wave. Type II gets in contact with and traps an erythrocyte with its pseudopod (1, 2). Its granuloplasm gradually fills the pseudopod so that it reaches the region where the erythrocyte is trapped (3). It follows a contraction at this granuloplasm which is observed in the margin of the cell as a constriction wave (4). The elongating wave (b) shows a circular contraction, and the shortening wave (a) a longitudinal contraction. And the erythrocyte assumes a pear-shaped appearance in the trapped region and becomes increasingly elongated (5). With an intensified development of the elongating wave the erythrocyte comes to be bisected (6).

The findings so far obtained of erythrophagocytosis facilitate general observations and they are common with phagocytosis for foreign bodies other than an erythrocyte, as reported later. They seem to afford a useful clue in explaining the dissociation between phagocytosis and the movement of leucocytes.

c) **Bacteria Phagocytosis:** The material employed was *Staphylococcus epidermidis et aureus*. In bacteria phagocytosis, as in erythrophagocytosis, first the pseudopod of neutrophils adheres to a bacterium and encloses the bacterium from the outside as it shows a progressive development. Then both tips of the pseudopod are fused together to make a phagocytic vacuole and engulfing is completed. Sometimes a mass of bacteria is engulfed at the sametime. Therefore, it does not seem reasonable to infer that bacteria may multiply within the cytoplasm only on the basis that bacteria are rather numerous found in the phagocytic vacuole.²¹⁾ On electron microscopic findings, it is observed that after the cell membrane of the pseudopod encloses a bacterium with its fused tips and its fused tip is separated from its surface, a bacterium is transferred into the cytoplasm without disappearance of the cell membrane. Thus it is revealed that the limiting membrane of the phagocytic vacuole is originated from the cell membrane.

The bacteria phagocytosis of monocytes and peritoneal macrophages is fundamentally the same as neutrophils (Photo. 2 i-v). In any case, after a foreign body is enclosed, an inactive cell transfers it into the cytoplasm as the pseudopod retracts, and an active cell transfers into such, it as the pseudopod proceeds. On

electron microscopic findings, no case has shown phagocytosis through the smooth surfaced endoplasmic reticulum (Photo. 3).

d) India Ink Phagocytosis^{27),29)}: On electron microscopic findings, the India ink phagocytosis of neutrophils and peritoneal macrophages is, as in bacteria phagocytosis, started off with the enclosing of India ink particles with the pseudopod, fusion of the tips of the pseudopod, formation of the phagocytic vacuole, separation of the vacuole from the cell membrane of the pseudopod through transference of the India ink particles into the cytoplasm (Photo. 4). Within the phagocytic vacuole, India ink particles show a trend of adherence to the vacuole wall.^{1),27)}

It disclosed that bacteria phagocytosis and India ink phagocytosis are fundamentally the same as erythrophagocytosis and that phagocytosis is satisfactorily carried out since cells can develop the pseudopod in various directions despite their decreased motile function. Hence there is a possibility of the development of dissociation between phagocytosis and the movement of cells.

e) Pinocytosis³⁸⁾: Those neutrophils which develop pinocytosis are, as already mentioned, those whose pseudopod has a low surface tension, a broad width, a feeble margin and many folds. The tips of the neighbouring folds are fused together to make a droplet. In transferring the thus formed droplet into the cytoplasm, an active cell keeps its pseudopod proceeding, while an inactive cell keeps its pseudopod retracting. On electron microscopic findings the membrane of the droplet is found to be of cell membrane origin (Photo. 5).

IV. Findings following Phagocytosis

Studies were made on the findings of engulfed *Staphylococcus epidermidis* et aureus and devouring human neutrophils and guinea pig peritoneal macrophages following phagocytosis.

The pinocytic droplet of neutrophils, when introduced into the granuloplasm, diminishes and is out of the scope of a phase contrast microscope in about ten minutes. But the phagocytic vacuole which has devoured bacteria grows bigger with the lapse of time, or sometimes it is fused with another vacuole to appear bigger,⁴⁷⁾ or occasionally it bursts to expose the trapped bacteria in the cytoplasm. If viewed with an electron microscope, the limiting membrane of the phagocytic vacuole becomes swollen, showing low electron density, and is partially interrupted. Then it utterly disappears. Sometimes it is found filled with the flowing of cytoplasm. There has been a report that around the limiting membrane of the phagocytic vacuole there exist organelles.^{11),20)} We, however, have never encountered such a finding.

The Staph. epid. in the phagocytic vacuole of neutrophils shows a decrease in electron density in its body. The decrease may be due to a swelling attributable to a decrease in the permeability of the cytoplasmic membrane of

the bacteria. Then an electron dense mass which constitutes the bacterial body loses its homogeneity resulting in an irregularity of electron density. The swollen bacterial cell wall bursts leading to destruction (Fig. 5). Even in *Staph. aureus*,

Fig. 5. Destructive process of *Staphylococcus epidermidis et aureus*

the same destructive process is found more than 15 minutes after phagocytosis is carried out. When peritoneal macrophages devour *Staph. epid.*, there follow changes in the bacterial body as is the case with neutrophils (Photo. 6). But when they devour *Staph. aureus* there follow no changes within 90 minutes.

The observation of neutrophils which have devoured bacteria with a phase contrast microscope reveals that the neutrophils remain active so long as the number of the engulfed bacteria is small and that they become less and less active as the number of the engulfed bacteria increased, being accompanied by a gelation of the cytoplasm. As the gelation progresses, the cell loses its activities resulting in fatality. The fatality occurs earlier in case *Staph. aureus* is devoured than is the case with *Staph. epid.*

SUMMARY

1. Phagocytosis is carried out in the following process : the cell membrane

of the pseudopod contacts and adheres to a foreign body ; it encloses the foreign body with its fused tips ; its fused tip portion is separated from the cell membrane of the pseudopod and transferred into the cytoplasm.

2. Development of the pseudopod is the prerequisite in phagocytosis. The pseudopod which show a favourable phagocytosis is the one whose cell membrane has a low surface tension and easy adhesiveness to a foreign body. When the cell is possessed with such pseudopod, pinocytosis is very likely to occur which enables the cell to engulf a foreign body including its medium without touching it.

3. The adhesiveness of a foreign body constitutes another important factor. Especially when a foreign body is of a big size its low adhesiveness has only a few chances of developing surface phagocytosis.

4. Phagocytosis is carried out only through the pseudopod of a cell. The pseudopod is what is pushed out of the easily liquidifying substance in the cytoplasm by contraction of an actomyosine like contractile protein which is considered to exist in the surface layer of the granuloplasm. The energy of this contraction is ATP which is also needed for the maintenance of surface tension and adhesiveness of the cell membrane. A decrease in the production of ATP results in a decrease in the activities, surface tension and adhesiveness of the cell membrane of the pseudopod.

It is concluded that phagocytosis is performed by a mechano-chemical system which is coupled by ATP.

5. A decrease in energy is not always proportionally accompanied by a decrease in phagocytosis. Occasionally an adverse relationship is observed, since phagocytosis is subjected to various interacting factors, such as the size, number, developmental speed and frequency, surface tension and adhesiveness of the pseudopod, and the nature of foreign bodies to be devoured.

6. Discussions are made of the findings of cells, phagocytic vacuoles and devoured bacteria following phagocytosis with a phase contrast microscope and an electron microscope.

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Photo. 1. Whole Erythrophagocytosis of Neutrophils.

Trapping of an erythrocyte with the pseudopod. An erythrocyte is surrounded by the pseudopod. The limiting membrane surrounding the erythrocyte continues to make the leucocytic cell membrane.

Photo. 2. i-v. Bacteria Phagocytosis of Human Blood Monocyte.

i. Contact to a bacterium with the pseudopod.

ii. The bacterium being enclosed by the pseudopod.

iii-iv. The bacterium transferred into the pseudopod.

iii →

← iv

v. The bacterium transferred into the granuloplasm.

Photo. 3.

Photo. 4.

Photo. 3. Bacteria Phagocytosis of Peritoneal Macrophages.

There are observed the bacteria surrounded by the pseudopod and bacteria existing inside the pseudopod and granulooplasm. The latter two are enclosed by the cell membrane.

Photo. 4. India ink Phagocytosis by Neutrophil.

Fusion of the tips of the pseudopod. The pseudopod traps India ink particles together with their medium. The tips of the pseudopod are seen to be in contact.

Photo. 5.

Photo. 6.

Photo. 5. Pinocytosis by Neutrophil.

Trapping of a medium with the pseudopod.

Photo. 6. Bacteria Phagocytosis of Peritoneal Macrophages.

The bacterium in the right of the lower phagocytic vacuole shows a decrease and a loss of homogeneity in electron density. The bacterium in the left shows an rupture in its cell wall, revealing an outflow of its contents. The bacterium in the upper phagocytic vacuole is destroyed.