Medical History, 1994, **38:** 91-101.

TEXTS AND DOCUMENTS

MAX SCHULTZE AND THE LIVING, MOVING, PHAGOCYTOSING LEUCOCYTES: 1865

by

DOUGLAS B. BREWER *

In his study, published in 1865,[1] of living human leucocytes examined at body temperature with the highest powers of the microscope then available, Schultze described for the first time four different types of leucocyte corresponding to what are now recognized as the lymphocyte, the monocyte, the neutrophil polymorphonuclear leucocyte, and the eosinophil leucocyte. The detail and precision of his descriptions of the movement of cells, and particularly his description of the intracellular granules and of the phagocytosis of a variety of particles, and their intracellular movements, are, to one who has examined these happenings with modern phase contrast microscopes, truly astonishing.[2] Yet his findings had little influence on current thought regarding the function of the phagocytes in inflammation. It was to be almost another twenty years before Ehrlich developed his staining methods which greatly simplified the recognition of the different types of leucocytes, and before Metchnikoff convincingly demonstrated the importance of phagocytosis in defence against bacterial disease.

The reasons for this are uncertain. The title of the paper is singularly uninformative. 'Ein heizbarer Objecttisch und seine Verwendung bei Untersuchungen des Blutes', i.e. 'The heated stage and its use in the investigation of the blood'. It gives no indication of the wealth

* D. B. Brewer, Professor Emeritus, Department of Pathology, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT.

I am grateful to Dr A. J. Howie, Department of Pathology, University of Birmingham, for reading this paper, and for his helpful suggestions. I am also grateful to Emeritus Professor P. Eckstein for his helpful comments on the original translation. Dr U. Zängl-Kumpf, of the University Library, Bonn, kindly sent me information relating to Schultze, in particular a photocopy of part of the thesis of R. R. Lüker 'Max J. S. Schultze (1825–1874) und die Zellenlehre des 19. Jahrhunderts' for which I am most grateful.

I have deposited the complete translation of Schultze's paper in the Barnes Library, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT, with also a complete translation of W. Preyer's paper (ref 20) and a partial translation of Friedrich von Recklinghausen's paper (ref 25), as well as a complete translation of Elie Metchnikoff's paper 'Ueber eine Sprosspilzkrankenheit der Daphnien' *Virchow's Arch.*, 1884, **96**: 177–94.

[1] M. J. S. Schultze, 'Ein heizbarer Objecttisch und seine Verwendung bei Untersuchungen des Blutes', *Archiv. f. mikrosk. Anatomie*, 1865, **1**: 1–42.

[2] J. E. Trowell and D. B. Brewer, 'Degranulation of chicken heterophil leucocytes during phagocytosis, studied by phase contrast and interference microscopy', *J. Path.*, 1975, **120**: 129–44.

of important and original observations contained in the 42-page article which was first published in *Archiv für mikroscopische Anatomie*, a new journal that Schultze had recently founded and was therefore not well-known.

The paper not only distinguishes for the first time the four different types of leucocyte; it also describes, at some length, the survival of white cells kept under a variety of conditions outside the body, the effect of heat on the leucocytes, and, at rather tedious length, the effect of heat on the red cells. Finally he describes what he calls "granule formation", which must be one of the first descriptions of platelets.

Another reason for the lack of an immediate impact of these findings was probably the fact that this investigation was to Schultze not primarily a study of leucocytes. He was interested in the concept of protoplasm as the basis of living matter and had investigated its nature in a very wide range of living organisms. In the opening paragraph of the paper he said that he was led to construct a warm stage as a result of the observations he had made on the effect of raised temperature on the cytoplasm of Rhizopoda and on the movement of the protoplasm of plant cells. He never returned to the study of blood cells. Almost all his publications after 1865 were concerned with the structure of the retina and with nerve endings in man and a wide variety of animals, including the crab, insects, and the lamprey.[3]

Max Johann Sigismund Schultze was born in 1825, in Freiburg im Breisgau, which lies between the Rhine and the Black Forest. His father was Carl August Sigismund Schultze, who was then professor of anatomy and physiology at the University of Freiburg. In 1830 his father moved to the University of Greifswald on the Baltic coast where Max Schultze was brought up in a cultured, academic household. Initially he was educated at home, and developed interests in music, natural history, and drawing that were to remain with him for the rest of his life. He studied medicine at Greifswald, and spent one year at Berlin. From 1850 to 1854 he worked as prosector and *privatdocent* with his father. From 1854 to 1859 he was professor at Halle. In 1859 he moved to Bonn as professor, where he worked until his death in 1874.

Although he qualified in medicine, his interests were exclusively in the scientific study of the structure of tissues, particularly the sense organs, the development of the concept of the cell, and the nature of protoplasm, especially its movement. As his pupil and obituarist G. Schwalbe[4] wrote, "In Easter, 1850, he was appointed as prosector in his father's anatomical institute, and could now dedicate himself to science and be disturbed no more by the demands of practical medicine". He was then aged twenty-five and had passed his state examinations in 1849/50.

His studies of protozoa and simple invertebrate forms, as well as animal cells, led him to reject the concept of the cell, based on plant structure, as a bladder-like structure with a membrane, contents and a nucleus. Instead he conceived protoplasm as the basic cell substance of both plant and animal cells and regarded the cell as a mass of protoplasm containing a nucleus. In developing this view he became convinced that the protoplasm of the cell had no covering membrane, that is, it was naked. This erroneous view was also shared by other contemporary scientists, amongst whom were Friedrich von Recklinghausen and William Preyer. But this view brought him into bitter conflict with Carl

[3] G. Schwalbe, 'Max Schultze, geb. 25. März 1825, gest. Jan. 1874', *Archiv. f. mikro. Anat. u. Entwicklungsmechanik*, 1874, **10**: i–xxiii, p. xxii.

[4] Ibid., p. vii.

B. Reichert, who eventually refused to publish papers by Schultze in the journal he edited, *Archiv für Anatomie, Physiologie und wissentschaftliche Medicin*. This refusal led Schultze to found the *Archiv für mikroscopische Anatomie*. According to Schwalbe the journal thrived during the ten years of Schultze's editorship, when it became "a rich treasure chest of manifold scientific treasures".[5] It has survived and prospered to this day, having undergone several changes of title; it now appears in English as *Cell and Tissue Research*.

Schultze suffered a number of personal tragedies in his life. In the autumn of 1865, at the prompting of his friends and colleagues worried that his heavy work load was having a deleterious effect on his health, he was persuaded to take a holiday with his wife at Ostend. Sadly she contracted typhoid and died, and the following year his two younger sons died.[6]

In 1868 he remarried and a happy and successful domestic life developed. His scientific career also prospered at Bonn and in 1869, after he had been Dean of the Medical Faculty for the year 1867–8, authority was given for a new department of anatomy to be built. Schultze devoted a great amount of time and effort to this, so much so that his research suffered. When the building was finished, as he had decided to stay in Bonn, he built a new house for his family. When it was finished, his friends and colleagues organized a surprise party for him. Schultze, with all the planning and building completed, was now looking forward to devoting all his energies to his research. Most tragically, he died suddenly a week later, on 16 January 1874, from a perforated duodenal ulcer, aged only forty-nine.[7]

THE WARM STAGE

The warm stage was made for Schultze by the mechanic Mr Geissler. The central part was about the shape and size of a normal microscope stage, on which it was placed. Two broad limbs extended out laterally for a short distance, and then extended forward at right angles. The stage was heated by a spirit lamp underneath these limbs and the temperature controlled by varying the distance along them at which the spirit lamps were placed. The temperature of the stage was measured by a thermometer which was wound tightly about the central hole in the stage. The stem of the thermometer extended forwards and upwards so that the temperature could easily be read. Schultze carefully calibrated the warm stage by observing the melting of wax in thin capillary tubes and in watery emulsions, the melting point of the wax having previously been determined.

He carefully claimed priority for the development of the warm stage, writing that he had demonstrated his warm stage to the Lower Rhine Society for Natural History and Medicine on 8 June 1864, whereas Alexander Rollet had presented his at a meeting of the Viennese Academy on 14 July 1864. In making this claim Schultze told an amusing anecdote. In the account of the meeting which was published in the *Berliner klinische Wochenschrift*, 1864, no. 36, he had written that the white cells move like amoebae between the red cells, but the printers had misread this as "Ameisen", the German word for ants. It appeared in print that Schultze's white cells moved like ants and, he joked, "That has led to the idea that my blood cells have six legs".[8]

[5] Ibid., p. xiv.

[6] R. R. Lücker, 'Max J. S. Schultze (1825–1874) und die Zellenlehre des 19. Jahrhunderts', Inaugural Dissertation, University of Bonn, 1977, p. 30.

[7] Ibid., p. 35.

[8] Schultze, op. cit., note 1 above, p. 9.

I now give a translation of the section of Schultze's paper dealing with his observations on living white cells. In the original paper the 41 pages run without a break. In my translation of the whole paper I have inserted headings, and I have kept them in this extract. I have not included some of Schultze's figures and so have had to change the figure numbers, but Schultze's original numbers in the legends are included, otherwise the translation follows the original text.

MY OBSERVATIONS ON WHITE BLOOD CELLS

The main object of my attention in observing the blood was first to examine the properties of the white blood cells. It can be accepted from their ability to move independently, as was first precisely learnt from the careful observations of Lieberkuhn,[9] that they might be influenced by the temperature difference of the surrounding medium, in the same way as the protoplasm of plant cells, as has been demonstrated by myself and others.[10] The successful results corresponded completely to expectations.

In a drop of fresh blood thinly spread under a coverslip with the temperature of the warm stage held at 36°–40 °C most of the white blood cells show such lively movement that the known slow movement of the same cells at the usual room temperature appear as a state of almost complete repose. The changes are nearly the equal of the lively movements of the small amoeba, commonly called Amoeba diffluens.

Not only are the changes in form much quicker than those at normal room temperature, but the character of the movements is different. At a temperature of 15°–20 °C we commonly observed an exceedingly slow and apparently aimless projection and withdrawal of processes, but at body temperature a movement of the whole cell is achieved. The white blood cells spread themselves out thinly over the surface of the coverslip and send out one or more processes in advance of themselves, as if exploring. Then the whole mass of the cell flows forward after the processes. Sometimes the part moving forward does not deserve the name of "process": rather it is the anterior part of the cell which drags the posterior part after it. Briefly, the white blood cells creep about between the red cells, now free, spread out on the surface of the coverslip, now pushing into a collection of red cells in order to clear a way through.

White cells in cooled blood are shining, strongly refracting cells, with a sharply defined contour and of spherical or nearly spherical shape. During movement they become spread out and thinned. They form plates of variable form with delicately defined peripheries. Here and there they push forward processes which often are pulled out into long threads, and which then return to the main mass of the cell. The finely granular substance of the cells, the protoplasm, is in continuous movement, changes in form going hand in hand with movement. In many of the white cells the strongly refractile granules within the cell can readily be distinguished individually, and at moderate magnification of about 400 times they give a clear picture of the changes inside the protoplasm during flowing movement. The nucleus, either single or multiple, can be followed in most white cells during this movement. Most nuclei are the same refractive index as the protoplasm and so can hardly be distinguished from it. With a good strongly magnifying lens (Zeis F, or Hartnack 9 and 10) one can often recognize the nucleus, even when it is not clearly defined, particularly in darkly granular cells where it can be seen as a bright spot, and here its movement from one end of the cell to the other, depending on changes in the shape of the cell, can be observed. How firmly the cells adhere to the glass surface,whilst they are moving, is evident from their resistance to the streaming of the plasma, in which the red cells continue to move. Often during observations on a warm stage, a lively streaming of plasma suddenly develops, probably due to an active evaporation from the edge of the coverslip. As a result, I saw the whole field of view in very active movement, but the moving white cells constantly maintained their positions.

[9] *Joh. Müller's Archiv* etc., 1854, p. 14. References 9 to 12 are from Schultze's paper and I give them here as he presents them in the paper. I have not translated the titles where they occur to indicate which references are in German.

[10] M. Schultze, *Das Protoplasm der Rhizopoden und der Pflanzen-zellen*, 1863, p. 46. W. Kühne, *Untersuchungen über das Protoplasm*, 1861, p. 100 ff. Juls. Sachs, *Flora 1864*, No. 3, p. 399, 49ff.

As is well known, all white blood cells are not the same type and they also show differences in their mode of movement. The differences, which even the most cursory observer of the blood cannot miss, are concerned with size. This varies quite significantly. The content of the nuclei has been discussed many times. They may be found to be large and simple or small and multiple. Finally, as emphasized by Wharton-Jones,[11] fine and coarsely granular white cells are found side by side in human blood and in the blood of most animals. This difference has only been superficially mentioned by later observers.[12]

In my blood and the blood of some other persons, I differentiate the following sorts of white cells. I begin with the smallest type, which does not reach the size of the red blood cells, and often is even considerably smaller, as a comparison with the red cells at the same magnification shows. They are spherical with a very delicate outer margin, with few granules and with refractile properties not very different from the surrounding fluid. Even without the addition of reagents, one can distinguish a large spherical nucleus surrounded by a very small amount of protoplasm. The smallest of these cells, which in my blood have a diameter of 0·005 mm, are exactly the same size as nuclei of the larger cells, which undoubtedly have a thin covering of protoplasm. I do not wish to claim that in these smallest cells the protoplasm surrounding the nucleus is missing, but the appearances can be attributed to an insignificant amount of protoplasm. In the larger of these cells I have often seen, without the addition of reagents, two plano-concave nuclei lying close together with the flattened surfaces touching each other. In both, the same sort of little nuclear body may be seen. The protoplasm in these cells is also arranged in a thin cover about single or double nuclei.

Because of the small amount of protoplasm, nothing precise can be said regarding its finer structure. Individually recognizable granules are mostly missing. The protoplasm shows a light cloudiness, which gives a hint of a granular contents. These white cells, which are smaller than red cells, show no movement or change of shape on the warm stage at body temperature (38°–40 °C).

This category of cells is immediately followed by a category of somewhat larger cells of about the usual diameter of red cells or slightly less. Considerably more protoplasm is present. The diameter of the nucleus is unchanged. At body temperature these cells show changes in shape. They put forth short, mostly pointed, processes, and retract them again. I saw no actual creeping movements. The protoplasm is exceedingly finely granular, but no molecular movement is seen.

In the third category we place the white blood cell which, according to previous descriptions, can be categorized as "the typical form". In the resting spherical state they measure 0·009–0·012 mm; their diameter therefore exceeds that of other white cells by a half (Figure 1a). In fresh blood taken from a vein they are seldom spherical: mostly, as in Figure 1, they show an irregular extended form. The granules in the protoplasm are extraordinarily fine, but molecular movement could not be detected. In exceptional instances one sees nuclei palely shining through. There may be one or two, or possibly more. When only a few nuclei are present they are the same size as those of the smaller white cells, but with an increased number of nuclei they are reduced in size. The activity that these cells display at body temperature is very surprising. As soon as the temperature of the drop of blood, by slow warming, has reached 35° and has increased to 38° or 40°, the movement begins which resembles that of a creeping amoeba (see Figure 2). The previously spherical, somewhat shining, strongly refracting body spreads itself on the surface of the coverslip and as a result at first has a paler contour. But the spreading does not take place evenly in all directions. It is as if the cell wishes to move in one direction, and to do this pushes forward a very pale, finely spiked mass, whilst the remaining part of the cell, still bright and more strongly refractile, slowly pushes after it, or the spreading may take place in several directions at the same time and will move forward on the finely spiked edge, adherent to the glass as long thread-like forms or wide plates. The movement of one cell process then gains the upper hand and the cell creeps forward, not disturbed by any streaming in the blood plasma, pushing between stationary or streaming red cells, forcing itself through narrow spaces and taking on all conceivably different shapes in rapid succession.

[11] *Philosophical Transactions*, 1846, 1, p. 82.

[12] G. E. von Rindfleisch has the credit for drawing attention to the constant occurrence of this modification in the white cells of the blood of the frog. He called them granular cells, *Experimentalstudien über d. Histologie des Blutes*, Leipzig, 1863.

In the resting rounded cells little can be determined of the fine structure of the protoplasm. The flattened, spread, moving cells provide a much better insight. With the highest magnification one can differentiate in the advancing cell processes with a finely spiky border, an almost hyaline cortex and a very pale finely granular interior. It appears as if there is no cell membrane. In the pale granular interior two apparently different sorts of granules can be distinguished.[13] One shines as if its refractive index was greater than the protoplasm, but only exceptionally was it so large as to appear to be limited as a ring, but it can be measured as large. The other sort of granule, which, I think, is more weakly refractile than the protoplasm, appears as larger and smaller round vesicles which look like holes in the protoplasm, i.e. they appear bright when the tube is either raised or lowered. These smallest vacuoles (I will describe them as such) are apparently the origin of a good part of the finely granular appearance in the blood cells under discussion. Undoubtedly, actual granules occur with them and this emerges amongst other things in the changes produced by the addition of water to these blood cells. It is known that as a result of slight swelling of clear spheres, changes appear in which there is lively molecular movement of the smallest granules inside the cell. In moving blood cells these appearances are not seen: the consistency of the living protoplasm is such that these changes do not occur.

While I think that one can differentiate between a thin, hyaline cortex and a granular interior in these cells, I am still some distance from accepting that there is a sharp boundary between these two areas. Such a boundary need not certainly exist as, in general, to every appearance, a membrane on the surface of these white cells is also missing. Whatever affects the nuclei themselves, so one would think, they must become more clearly recognized as the cells become flattened during movement. But this is not so in many cases. When I have been repeatedly successful in detecting two or three nuclei inside cells, their boundaries are always very pale and sometimes very doubtful; in other cases all trace of a nucleus is missing. It would, however, be rash to conclude from this that the nuclei are absent. We already know that if the refractive index of the nucleus hardly differs from that of the living protoplasm then the thicker the layer of protoplasm surrounding the nucleus the more difficult it is to distinguish the nuclei. Now it happens often enough in the moving blood cells that in addition to the pale, hyaline, creeping extended cell processes, there is a thicker brighter part of the cell body in which the nucleus may escape the notice of the observer. Thinning of the blood with weak acetic acid leaves no doubt that, at least, the greatest proportion of the white cells certainly contain one or more nuclei.

We have given here a demonstration of the behaviour of the common form of white cell. It remains for us to discuss the not uncommon differences in the remaining white cells. In agreement with Wharton-Jones I will call them "coarsely granular" in contrast to the previously described "finely granular". I have found in my blood and in the blood of other people scanty numbers of white cells which may be clearly distinguished from other cells by considerable numbers of small, strongly refractile, round granules, which have the brightness of small fat droplets, (compare Figures 4 and 5). When still the rounded forms are similar in size to the finely granular white cells, i.e. they are a little larger than the red cells. The dark granules can often be clearly distinguished in the resting cell, in which it can be recognized that molecular movement cannot be observed. The granules can be more clearly distinguished when the cells begin their creeping movement at a temperature of about 38 °C. Already at room temperature one can see a movement similar to that described by Lieberkuhn in finely granular white cells, that is a changing of form in which the cell processes are slowly extended and withdrawn. If one increases the temperature to that of the body a very much quicker movement develops with striking changes in form (Figure 5) and a more rapid amoeba-like movement develops with movement from place to place as we have just described in the finely granular white cells.

[13] The appearance of the neutrophil polymorph moving forward with the hyaline granule-free leading edge (Figure 3) is typical of polymorphs moving on glass surfaces. This leading edge has been referred to by W. S. Ramsay ('Locomotion of human polymorphonuclear leucocytes', *Exp. Cell Res.*, 1972, **72**: 489–501, p. 489) as the lamellipodium. It is difficult to interpret Schultze's description of two different sorts of granule in the finely granular white cell. Initially two sorts of granules were recognized, azurophil and specific granules. More recent studies have shown a marked variation in a considerable number of enzymes as between various granules, but currently there is no general agreement as to the details of a classification, see L. A. Boxer and J. E. Smolen, 'Neutrophil granule constituents and their release in health and disease', *Hematology/Oncology Clinics of North America*, 1988, **21**: 101–34.

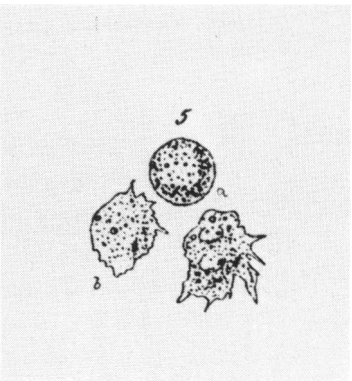

Figure 1: Large finely granular white blood cells. (a) sperical. (b) spiky (Schultze, Fig. 4).

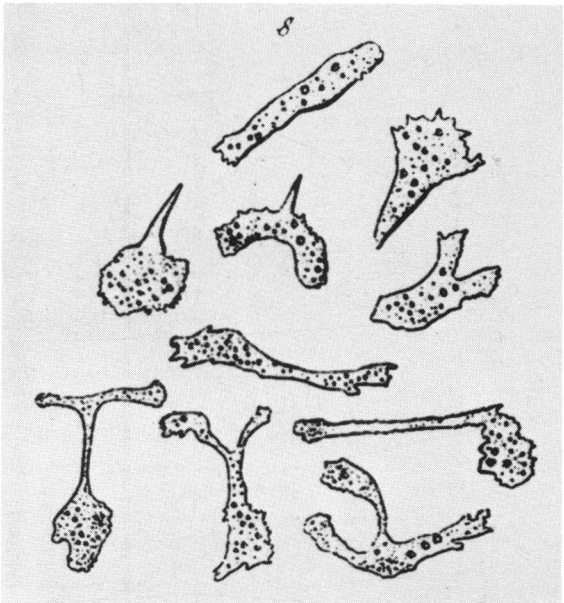

Figure 2: A finely granular white cell at 38° C on the warm stage, showing lively creeping movement. The shapes drawn show the same cell in a series of rapid changes in shape following one after another (Schultze, Fig. 8).

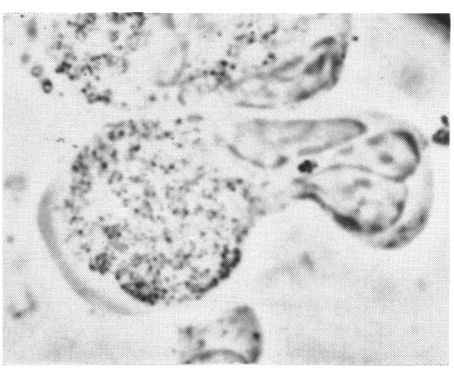

Figure 3: One of my neutrophil polymorphs filmed at 37° C by phase contrast microscopy. It is moving to the left. There is a granule-free lamellipodium at the anterior end of the cell. The main body of the cell is filled with granules of varying densities and the nucleus is at the rear. Magnification approximately ×2,500.

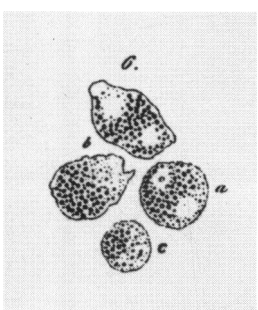

Figure 4: Large coarsely granular white blood cells. (a) sperical. (b) spiky. (c) small form (Schultze, Fig. 6).

Figures 1, 2, and 4–7 are Schultze's drawings slightly retouched. They are all of human blood cells and Schultze described them as all being drawn at the same magnification of 7–800 times. The legends to these figures are those of Schultze. I have included the original figure numbers in the legends.

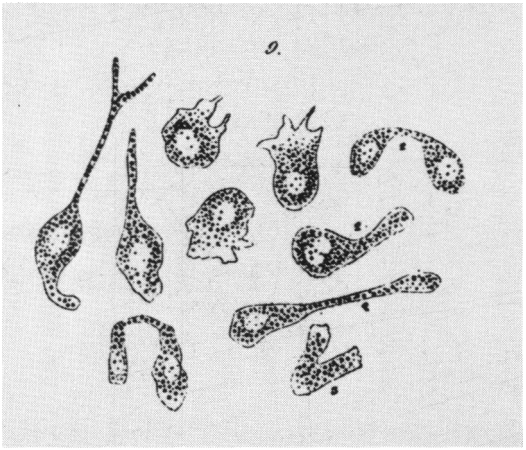

Figure 5: Coarsely granular white cells drawn in creeping motion at 38°C. The unnumbered cell with one nucleus and the cell numbered 2, with two nuclei, show successive changes in each of two different cells (Schultze, Fig. 9).

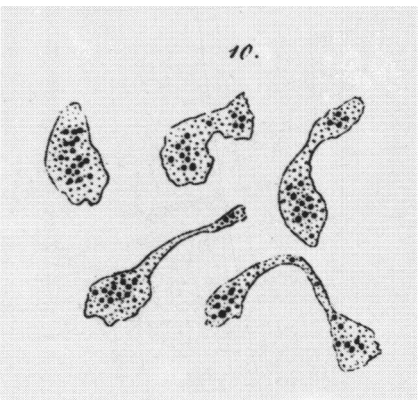

Figure 6: Finely granular white cells which have taken up cinnabar granules at body temperature (Schultze, Fig. 10).

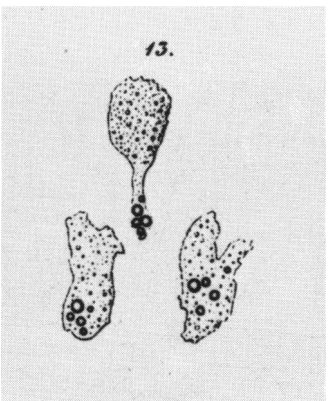

Figure 7: A finely granular white cell from a drop of blood diluted with milk. At body temperature it has taken up 5 droplets of milk (Schultze, Fig. 13).

These coarsely granular white cells form a much better object than the finely granular white cells, not only for studying the movement of the whole cell, but also for studying the interior of the protoplasm. The observation of the finely granular white cells on a warm stage, even when the most powerful and best lenses are used (Zeis F, Hartnack 10), is a task which for a satisfactory conclusion requires very favourable lighting and is a strain on the eyes. An immediate, important advantage of the observation of the coarsely granular white cells is that during their change in shape, at the same time, the movement of the granules inside them can be clearly followed. The slow flowing of the granules or the sudden rush resulting from the rapid projection of broad cell processes give a clear picture of the movements which the protoplasm of these cells undergoes. This is specially so in the long extended threads of protoplasm as are present in the moving cells drawn in Figure 5. The even flowing movement of the granules is very prominent, particularly because of the striking transparency of the protoplasm and the small number of granules. Often only a row of granules is seen in the protoplasm, particularly when the white cells push their way between many red cells, rather than when they are moving freely in the blood plasma. The movement of the granules in these long processes is commonly not to and fro, as I particularly emphasize here in comparison with that in the Rhizopods or pseudopods, but they move in one direction, following the movement of the protoplasm. There are certain differences in the form of the movement and the shape of the cell processes between the coarsely granular and finely granular white cells. Whilst with finely granular cells the spreading of the cell substance seems almost like a dissolving in which the finely pointed boundary of the cell, of almost disappearing thinness gives rise to thoughts that, in general, a sharp boundary has ceased to exist here, but the creeping movement of the coarsely granular cell shows a sharper, more rounded limiting boundary. The protoplasm here shows all the appearances that are more consistent with a resistant substance, at least on the surface. This is the same during movement as in the finely granular white cells, in which, in general, the changes in form follow each other just as rapidly and the same variation takes place inside the cell processes.

As Figure 5 shows, in the coarsely granular white cells the cell nucleus is mostly quite clearly perceived, admittedly not always sharply defined, rather less so than in the finely granular cells, rather as a clear spot in the coarsely granular cell substance produced by a displacement of the granules. The number of nuclei is 1 or 2. Their position varies. If two nuclei are present they can be close together or lie at opposite ends of the cell.

As I, like Wharton-Jones, differentiate fine and coarsely granular white cells in human blood, so I must add that intermediate forms between them occur. But these intermediate forms are found much more seldom than the extremes and the differentiation of the two types is fully justified. One may call cells intermediate forms which are by appearance and behaviour finely granular, but contain a few refractile granules of the sort found in the coarsely granular cells. They occur in different sizes.

PHAGOCYTOSIS OF PARTICULATE MATERIAL

Following the observations of the unexpectedly lively creeping movements which the white cells of human blood perform when warmed to body temperature and their constant similarity to certain of the delicate forms of amoebae, the question must immediately be posed whether it is possible to observe the uptake into their protoplasm of solid material as seen in feeding amoebae. A positive answer must probably be expected as it has been repeatedly observed that white cells and lymph cells in the blood of cold blooded animals constantly take up particles of dye into their protoplasm after the blood has been artificially mixed with the dye. E. Haeckel, through the opportunity of a memorable stay in the Mediterranean for a study of the natural history of Rhizopods, undertook a series of experiments on molluscs and crabs in which he was able to demonstrate, most convincingly, the uptake of dye particles by the white cells of the blood, both inside and outside the blood stream.[14] Then came the studies of von Recklinghausen[15] on the lymph cells and white cells in the frog and later the studies of Preyer[16] on the white cells of the same animal *in vitro* in which the uptake of milk globules and dye

[14] *Die Radiiolarien*, Berlin, 1862, p. 104. References 14, 15, and 16 are from Schultze's paper and I give them as he presented them.

[15] *Die Lymphgefässe und ihre Beziehung zum Bindegewebe*, Berlin, 1862, p.22.

[16] *Virchow's Archiv*, Bd. XXX, p. 420.

particles into the interior of these cells was observed. Because of the sluggish movements of these cells outside the body, on the microscope stage, the uptake of the dyes into these cells is only observed after great perseverance. How much more appropriate in this connection must be the rapidly creeping white cells at 30°–40 °C in warmed human blood. These assumptions have been confirmed by observations.

I first mixed a trace of finely divided carmine dye with a drop of fresh blood taken from the finger and observed it at 38 °C. After only a very few minutes I saw that most of the creeping cells were dragging a few particles of carmine with them. I saw such particles, with the elementary granules of the protoplasm, move from one process of the cell to another. I saw how they were dragged along in the long drawn-out threads of protoplasm, and how they were retracted with them. Sometimes they lay together in larger clumps, sometimes they were divided amongst the cell processes, following the form and inner movement of the cell so that not the slightest doubt remained that the carmine was actually taken up into the protoplasm and was not simply adhering to the sticky surface of the white cells.[17]

Instead of carmine, I have used a few other dyes, namely cinnabar, indigo and aniline blue, and also milk, with similar, and to some extent, better results. The granules of cinnabar are mostly finer than carmine and are taken up more quickly and in greater amounts, so that the movement of the granules of the dye could be observed in the protoplasm of the creeping cell with the greatest clarity (Figure 6). Aniline blue, in its finest granules, has a more intense and prettier colour than indigo. It is very finely distributed in the blood and so, all things considered, is as readily taken up into the cells as indigo.

As differences have been seen in the type of locomotion and in the form of the advancing creeping cortical processes between the finely granular and the coarsely granular white cells so we have also noticed differences in relation to the dyes. In general, the coarsely granular white cells are much less inclined to take up foreign material than the finely granular white cells, which we may be justified in accepting as obviously depending on the consistency of the cortex of the protoplasm. The coarsely granular white cells do take up granules of dye, as I have observed in experiments with aniline blue, amongst others. One such cell, containing a few blue granules, is illustrated in two different forms. It was quite striking to me that the liveliness of the movement was obviously much reduced following the uptake of dye particles, an appearance that I often met with the finely granular cells.

The moment in which the dye molecules are taken up into the interior of the protoplasm does not appear to be marked by any specially conspicuous manoeuvre. I have never seen any special processes which the cell has extended to cope with foreign granules. What I have observed can be limited to the appearance that during the even forward creeping movement the dye is forced into the interior of the protoplasm, as if by pressure. It might be suspected that there might be a special place on the surface of the cell for the uptake of foreign material. This possibility, however, is somewhat improbable, and observations do not support it.

From observations of the creeping cells, the properties of the cell surface seem evenly distributed all over the cell. It should be mentioned that, commonly, after mixing a drop of blood with finely powdered dye, a certain part of it adheres to the well-known sticky, slimy surface of the white cell. Then on the first creeping movement of the cell on the warm stage the dye, in part or whole, is immediately taken into the interior of the cell, so in this case the observation of the moment of "devouring" as a separate phase was not achieved.

The experiments with milk are of special interest in that the milk droplets that are taken up are much larger than the dye particles. Diluting the blood with two thirds its volume of fresh cow's milk, as I have done, produces no noticeable change in the formed elements of the blood. On the warm stage the white cells move with the same speed and they remain alive as long as in undiluted blood. As soon as creeping movement begins one sees white blood cells with smaller and larger milk globules adhering to them. The smaller and middle-size droplets, with an average diameter of 0·003 mm, very soon

[17] Schultze, op. cit., note 1 above, pp. 9–18.Here (pp. 18–19.) Schultze described, at some length, techniques to obtain very thin preparations in which the process of phagocytosis could be observed more precisely, firstly by diluting the blood with amniotic fluid to which tincture of iodine had been added, and secondly by obtaining a very thin film of blood between a slide and coverslip.

appear inside the protoplasm of the cells and there, with the intrinsic granules, move to and fro. In Figure 7 I illustrate a white cell which contains five different sized milk globules. When I saw these first they were in a little clump in a cell process and then they were pulled into the body of the cell during creeping movement. This cell process was withdrawn and the milk globules moved near to the centre of the cell where they rapidly interchanged their positions in the flowing protoplasm of the cell as it moved forward.

The observations of creeping white blood cells in different stages of movement convinces us that the protoplasm does not have a differentiated outer layer, a cell membrane in the sense of the old school. The successful feeding experiments with dyes, and with milk droplets, naturally, contribute only to the confirmation of this opinion. The observation of the creeping forms shows no hint of a membrane. The physical properties of the surface speak most decisively for naked protoplasm. The appearances of the uptake of foreign material into the interior of the cell at any place on its surface would, assuming the existence of a membrane in which we could see no large opening, be a paradox and would speak against all analogies. Under these circumstances there cannot be a moment's doubt that I consider the human white blood cells to be membrane-less cells, which from my observations, recorded in different places, consist only of protoplasm, with an included nucleus.[18]

The effect of body temperature on the activities of the white cells is very vividly conveyed by Schultze's description of the transformation from "almost complete repose" to the actively moving and phagocytosing cells. This is in great contrast to other descriptions. Preyer in his studies of phagocytosis[19] used white cells from a variety of amphibia and he wrote of phagocytosis, "I only succeeded in observing the uptake of material after observing a suitable amoeboid cell, on which indigo particles were lying, for many hours".

I find Metchnikoff's assessment of the significance of Schultze's work rather inadequate.[20] He wrote that Schultze showed that there are several different kinds of leucocytes, and that an exact knowledge of the different forms dates only from the discoveries of Ehrlich. Ehrlich himself[21] commented that after Schultze had shown that there were recognizably different types of blood cells, research in this field had not advanced and the confusion was such that Rindfleisch complained that the white cells appeared to be a sort of "omnibus" in which all sorts of possibilities travelled.

From the point of view of the function of the white cells, the work of Schultze was more important than that of Ehrlich; not only did he distinguish four different sorts of white cells, but he demonstrated important differences in their movement and their phagocytic abilities. Only the two types of granular cells showed any important motility or phagocytic ability and the finely granular white cell was more motile and more actively phagocytic. This difference between the behaviour of the finely granular white cells, i.e. the neutrophil leucocytes, and the coarsely granular white cells, i.e the eosinophil leucocytes, moving on glass surfaces *in vitro* has been confirmed in recent modern studies.[22]

Ehrlich's staining methods, performed on fixed blood films, were simple to perform and permitted an easy recognition of the different types of blood cells, which correlated well with

[18] Ibid., pp. 19–22.

[19] W. Preyer, 'Ueber amöboide Blutkörperchen', *Virchow's Arch.*, 1864, **30**: 417–41, p. 420.

[20] Elie Metchnikoff, *Lectures on the comparative pathology of inflammation*, New York, Dover Publications, 1968, p. 113.

[21] P. Ehrlich, 'Methodologische Beiträge zur Physiologie und Pathologie der verschiedenen Formen der Leukocyten', in F. Himmelweit (ed.), *The collected papers of Paul Ehrlich*, London and New York, Pergamon Press, 1956, pp.124–9, on p. 124.

[22] G. T. Archer and J. G. Hirsch, 'Motion picture studies on the degranulation of horse eosinophils during phagocytosis', *J. Exp. Med.*, 1963, **118**: 287–93, pp. 288, 290.

the results of Schultze, but provided no direct evidence of the function of the different types. In fact, Ehrlich's studies[23] were really more haematological and there is no mention of phagocytosis. He was much preoccupied with a dispute with Robert Altmann over the nature of cytoplasmic granules in general, and of the cytoplasmic granules of the leucocytes in particular.

It is a little surprising that such an acute, critical observer as Schultze held so firmly to the erroneous belief that cells, in general, and white cells in particular, have no surface membrane, but it was a view widely held at the time. Both von Recklinghausen (1863)[24] and Preyer (1864)[25] thought that the ready uptake of foreign material into the white cells was convincing evidence for the absence of a cell membrane. Preyer described the white cells as "naked little bodies of protoplasm in which there are usually one or more nuclei clearly visible".

These early observers of the process of phagocytosis evidently thought of cell membranes as a barrier, but, as is now understood, the cell membrane is a complex and subtle regulator of what goes into and out of the cell, a complex mosaic of transporting molecules, receptors, and regulating molecules embedded in, and in many cases, bridging the bimolecular lipid membrane. The first stage in phagocytosis is the specific binding of the bacterium to the cell membrane. This binding results from interaction of the opsonising molecules (IgG and C3b molecules) on the surface of the bacterium with specific receptors in the cell membrane. The area of adhesion gradually increases until the bacterium is completely surrounded. It then separates from the surface membrane and moves into the cytoplasm of the neutrophil still tightly surrounded by the cell membrane.[26] A vacuole is then formed by the membrane becoming detached from the surface of the bacterium.

Another surprising feature of Schultze's study was that it made so little impact on the understanding of the role of the white cells in general and the part they play in the process of inflammation in particular. In recent times his work has been widely ignored. Thus it is not referred to in Herrlinger's (1956)[27] review of the historical development of the concept of phagocytosis or in Tauber and Chernyak's (1991)[28] more recent and much more extensive history of the origins of immunology. However, Rather (1972)[29] refers to it at some length.

At the time and for some years afterwards there was considerable confusion generally about the nature and origin of the cells found in the tissues in inflammation. It was widely held that what were called the mucus globules, the purulent globules, and the blood corpuscles were different cells, but there was convincing evidence to the contrary from

[23] P. Ehrlich, 'Histology of the blood', in Himmelweit, op. cit., note 21 above, pp. 181–268, on p. 234.

[24] F. von Recklinghausen, 'Ueber Eiter- und Bindegewebskörperchen', *Virchow's Arch.*, 1863, **28**: 157–97, p. 184.

[25] Preyer, op. cit., note 19 above, p. 420.

[26] F. M. Griffin, J. A. Griffin, J. E. Leider and S. C. Silverstein, 'Studies on the mechanisms of phagocytosis. 1. Requirement of circumferential attachment of particle-bound ligands to specific receptors on the macrophage plasma membrane', *J. exp. Med.*, 1975, **142**: 1263–82, pp. 1275–8.

[27] R. Herrlinger, 'Die historische Entwicklung des Begriffes Phagocytose', *Ergeb. Anat. Entwicklungsgesch.*, 1956, **35**: 334–57.

[28] A. I. Tauber and L. Chernyak, *Metchnikoff and the origins of immunology*, New York and London, Oxford University Press, 1991.

[29] L. J. Rather, *Addison and the white corpuscles*, London, Wellcome Institute, 1972, p. 211–12.

several different studies. Some years previously Waller (1846),[30] in a study that was largely ignored, demonstrated very convincingly that the white corpuscles of the blood emigrated from the capillaries into the tissues where they became the cells that were recognized as mucus or pus globules. Von Recklinghausen (1863)[31] in a series of well planned experiments examined the movement of what he called pus cells in the tissues. He induced pus cells in the lymph sac of the frog to phagocytose cinnabar granules and then placed fragments of cornea from a variety of animals in the lymph sac. After leaving the fragments in the lymph sac for some time, he was able to see that the pus cells, which appeared red from the cinnabar granules, had moved considerable distances into the corneal fragments and so he concluded that the pus cells produced by inflammatory stimulation are able to move very long distances through the tissues.

But the full significance of Schultze's demonstration of very active phagocytosis by the "finely granular cells" could not be understood until it was appreciated that in the circumstances of acute inflammatory bacterial diseases what was being phagocytosed and so destroyed were the pathogenic bacteria. The realization of the importance of bacteria dawned slowly and only became clear after the classical studies of Koch.[32] The slow and sometimes confused development of ideas in this field is well described by Tauber and Chernyak (1991).[33]

[30] Augustus Waller, 'Microscopic observations on the perforation of the capillaries of the blood, and on the origin of mucus and pus-globules', *Phil. Mag.*, 1846, **29**: 397–405, p. 398.

[31] Recklinghausen, op. cit., note 24 above, p. 189.

[32] R. Koch, *Investigations into the aetiology of traumatic infective diseases*, London, The New Sydenham Society, 1880.

[33] Tauber and Chernyak, op. cit., note 28 above, pp. 101–33.