

A disease of *Daphnia* caused by a yeast.
A contribution to the theory of
phagocytes as agents for attack
on disease-causing organisms

1884 • *Elias Metschnikoff*

Metschnikoff, E. 1884. Ueber eine Sprosspilzkrankheit der Daphnien. Beitrag zur Lehre über den Kampf der Phagocyten gegen Krankheitserreger. *Archiv f. pathologische Anatomie und Physiologie und f. klinische Medicin*, Vol. 96, pages 177–195.

THE COMMON WATER FLEA OR DAPHNIA seems quite suitable for studies on pathological processes and may be able to throw some light on many general questions in medicine. Although these crustaceans, because of their small body size and delicate nature, have proven to be quite unsuitable for the production of all kinds of artificial diseases, they offer many advantages

for the study of those disease phenomena with which they become afflicted artificially. Because they are relatively small and fairly transparent animals, they can be observed without damaging them for many hours at a time, and also repeatedly from day to day. . . .

The disease which I wish to describe in the following lines is a disease due

to a budding fungus, or loosely, a yeast. So far as I know, this disease has not been described earlier and was even unknown to me two years ago when I described another disease of *Daphnia*. I first found it last fall in an aquarium in which *Vallisneria* and *Daphnia* were almost the only flora and fauna. I noticed many *Daphnia* which seemed to be ill, and under the microscope could see that this was a different disease than I had seen earlier. The whole body cavity up to the last antenna was filled with a massive accumulation of fungus cells, which I demonstrated to be different stages of a single species of fungus. I have named this fungus *Monospora bicuspidata*. . . .

From the characteristics of this parasite, it seems to be very similar to the ordinary yeasts, although it is not possible to ascertain its definitive place in the system of fungi, since we know from the recent work of Brefeld that yeastlike stages occur in many different fungi (*Ustilago*, *Tremella*, etc.). . . .

I have observed all of the stages of the *Monospora* in the abdominal cavity of sick *Daphnia*. [See Plates IX and X] In the early period of the disease, one sees predominantly the budding conidia, while in the later stages the ascospores prevail. In spite of many experiments, I have not yet been able to cultivate this fungus on artificial media. I have tried various nutrient media such as acidified meat extract, orange juice, and so on.

In the individuals dying of the disease, a large number of spores in asci are produced, which are consumed by healthy individuals. Although the asci do not rupture in water, the spores which occur in the intestinal canal are mostly free of the asci, which I believe is due to the action of the digestive juices of the *Daphnia* on the asci. As

a result of peristalsis, the spores penetrate the intestinal wall, so that they are partly in the intestine and partly in the body cavity. The most favorable spores for observation are those which are partly in the body cavity, but with most of the spore in the intestinal wall and intestinal cavity. Hardly has a piece of the spore penetrated into the body cavity, than one or more blood corpuscles attach to it, in order to begin the battle against the intruder. (Footnote: The blood corpuscles of *Daphnia*, like most vertebrates, are colorless, amoeboid cells which are adapted to the uptake of solid particles. They circulate in a system of cavities and are kept in circulation by a tubelike heart. *Daphnia* are completely lacking in blood vessels, except for a short outlet tube which several authors have called the aorta.) The blood cells fasten so tight to the spore that they are only seldom broken free by the blood stream. In this case, they are replaced by new blood cells, so that in most cases the spore is more or less completely surrounded by them. Often the spores penetrate completely into the body cavity, in which case they are even more likely to fall prey to the blood corpuscles. The number of blood cells which collect around one spore varies considerably. When many spores are in the body cavity at the same time, such a large number of blood cells surround them, that the whole area appears highly inflamed, so far as one can speak of inflammation in a vessel-less animal. The blood cells collected around the spore do not always maintain their individuality, and may unite occasionally into a more or less extensive plasmodium (a so-called giant cell). . . .

In the intestinal contents or excrement, one finds that the majority of the spores are intact, which indicates that they are unaffected by the diges-

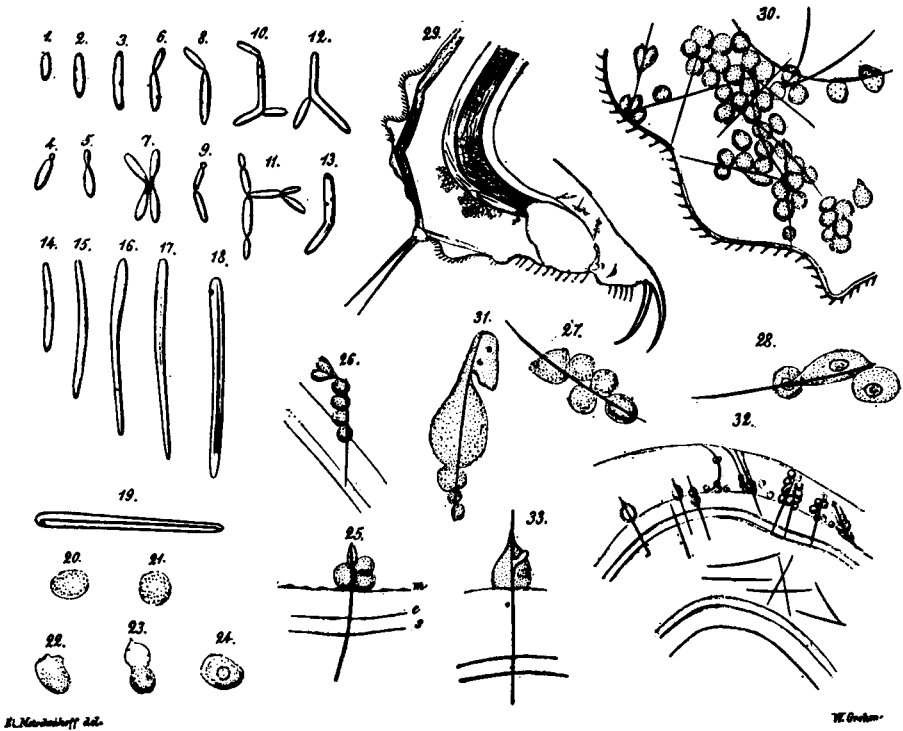


Plate IX

Figs. 1-14. Conidia of *Monospora* in various configurations. Figs. 15, 16. Elongated conidia, just before spore formation. Figs. 17-19. Formation of the ascospore. Figs. 20-23. Blood cells of *Daphnia magna*, drawn from life. Fig. 24. A blood cell treated with acetic acid. Fig. 25. A spore that has penetrated the intestinal wall, surrounded by four blood cells. (*m*) Muscle layer of the intestine, (*e*) Epithelial layer, (*s*) Layer of rods. Fig. 26. Another spore, as in Fig. 25. Fig. 27. A spore surrounded by blood cells from the body cavity of a *Daphnia*. Fig. 28. Another spore after treatment with acetic

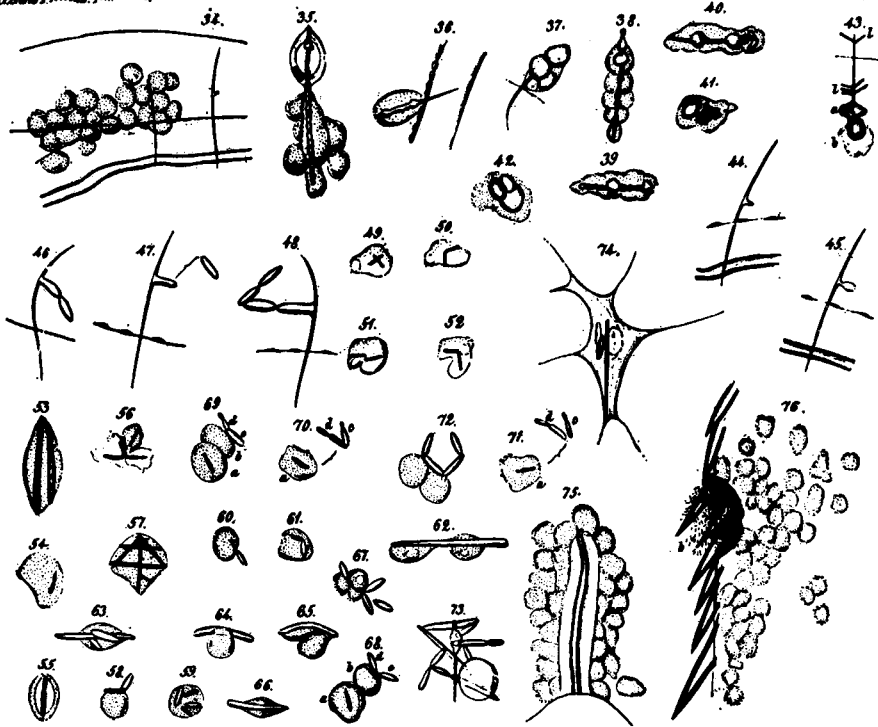
acid. Fig. 29. The abdomen of an infected *Daphnia*, with many spores in the body cavity surrounded by blood cells. Many spores are also seen in the intestinal wall and in the intestinal cavity. Fig. 30. Area of the abdomen of another *Daphnia* with intense accumulation of phagocytes around the spores. Fig. 31. Blood cells that have coalesced around a spore. Fig. 32. An area from the anterior portion of the body, with many free spores and engulfed spores. Fig. 33. A germinating spore and an adherent blood cell.

tive juices. Those that are surrounded by blood cells behave completely otherwise. After a spore has lain for a time in the middle of a number of these cells, it begins to undergo quite regular changes. First it thickens, turns light yellow in color, and its contours become jagged. Then it swells in several places to various sizes, assuming

round or irregular shaped balls, which become brownish yellow. Meanwhile the rest of the spore, which is still rod-shaped, seems lighter and yellower. Still later the whole spore comes apart into irregular, brownish yellow, dark brown and almost black grains, some large and some small. The connection of these particles to

Vizshin's *A.-bia.* Pl. XVI.

1897.



Metschnikoff del.

W. P. Adams sc.

Plate X

Fig. 34. An area from the posterior portion of another *Daphnia*. Figs. 35-42. Spores in various stages of alteration due to the action of blood cells. Fig. 43. A spore partially penetrating the wall: (a) small wall opening; (b) the lower portion of the spore, engulfed by a blood cell and markedly altered; (1) young *Leptothrix*, which has settled on the free portion of the spore. Figs. 44-48. Various stages of spore germination and conidia formation. Figs. 49-52. A single blood cell in four different configurations. Figs. 53-57. Various blood cells and conidia. Fig. 58. A blood cell adjacent to two conidia. Fig. 59. The same cell as in Fig. 58, one-half hour later. Figs. 60-66. Various blood cells and conidia. Fig. 67. A ruptured blood cell, from which the conidia have escaped. Fig. 68. Two blood cells, in one of which (a) a germinating conidium

rests, while in the other (b) two conidia (c, d) remain in contact outside. Fig. 69. The same picture as Fig. 68, one-half hour later. The conidium (d) has begun to form a bud. Fig. 70. The same as Fig. 69, without blood cell (b), which has in the meantime moved away, two hours later than Fig. 69. Conidium (c) is beginning to bud. Fig. 71. The same picture, one and one-half hours after Fig. 70. Fig. 72. Two blood cells adjacent to four conidia. Fig. 73. A group of conidia which have brought about the dissolution of a blood cell that had engulfed a spore. All that remains is an empty shell and fine debris. Fig. 74. A fibrous phagocyte containing three fungus cells. Fig. 75. An injured layer of tissue with many blood cells attached. Fig. 76. A disrupted area of another *Daphnia*, also with many blood cells.

the earlier delicate spore can only be determined through knowledge of the whole process of transformation of one into the other. In the meantime

the blood corpuscles have united into a fine-grained, pale plasmodium, which still has the ability to move by amoeboid motion. Occasionally one can

find in certain places in the *Daphnia* body, whole heaps of these plasmodia, which are especially striking because of the grains which they contain. . . .

I believe that the changes which take place in the spore are the results of the action of the blood cells. This belief is based on the following observations. When a spore remains for a long time with half of it in the intestinal wall and only half ingested by the blood cell, only this latter part undergoes the regressive changes and becomes definitely decomposed, while the portion lying in the wall maintains completely its normal appearance. Such examples are too frequent to make one doubt of their generality. . . .

From what has been said above, it is evident that spores which reach the body cavity are attacked by blood cells, and—probably through some sort of secretion—are killed and destroyed. In other words, the blood corpuscles have the role of protecting the organism from infectious materials. This does not always occur. In cases in which a large number of spores reach the body cavity, or for some other reason one or more spores remain unaffected by the blood cells, the disease may break out. . . .

Because the process described here can be observed much more favorably than the battle of phagocytes against bacteria, I will make a few additional comments on the observations. In order to obtain certain results, one must observe one organism for many hours. Then one can see that the blood cells really ingest the spores. Sometimes this process occurs very quickly, but other times it is a very slow process. . . . The number of spores which one blood cell can ingest varies; ordinarily one finds only two spores in each cell, but occasionally one can find three, four, or more spores.

The blood cell which ingests a par-

asitic spore still retains its ability to move. . . . Occasionally spore-containing cells unite together into a small plasmodium, which then harbors more parasites than usual. . . .

The blood cells are able to attack living fungus cells as well as the spores. . . .

Although it is true that the fungus cells are destroyed by the blood cells, on the other hand, it can not be denied that the blood cells can also be affected by the parasites. Several times I observed a blood cell full of parasites rupture before my eyes, setting the fungus cells free again. Also I could see a number of times that blood cells in the neighborhood of a large number of fungus cells would gradually dissolve and completely disappear. This indicates that the fungus cells produce a substance which is deleterious to blood cells. . . .

The farther along the disease has progressed, the more blood cells that are dissolved, so that at the time when the *Daphnia* contains a significant number of ripe spores, it reveals only a few or no blood cells.

Aside from the blood cells, only the isolated connective tissue cells play a similar role as phagocytes (eating cells). They behave in the same way as the blood cells in the ingestion of fungus cells. In the same way, they are dissolved by the fungus cells, so that in the later stages of the disease, all of the phagocytes of the animal body disappear. Other tissue elements do not suffer such a remarkable change. One may see a large number of fungus cells develop on the heart muscle, but the heart continues to contract regularly. . . .

When the *Daphnia* has once become sick and has produced fungus cells in it, it generally dies without a chance of recovery. In the last period of the disease, so many spores have been

formed that the body takes on a diffuse milk-white color. . . . The whole disease takes two weeks to proceed. A young isolated *Daphnia* which had just begun to form fungus cells from spores died 16 days later. . . .

From the above it can be seen that the infection and illness of our *Daphnia*s is a battle between two living beings—the fungus and the phagocytes. The first consist of one-celled lower plants, while the latter represent the lowest tissue element and have a great similarity with the simple organisms (*Amoeba*, *Rhizopodium*, and so on). The phagocytes, which have retained the primitive property of taking up solid food, can act because of this as destroyers of parasites. They seem therefore, as the bearers of nature's healing power, which has been known to exist for a long time, and which Virchow first placed in the tissue elements. The whole course of the *Daphnia* disease fits in with the basic thoughts of this master of cellular pathology, all the more so since the main role has here been found to be an independent cellular element. . . .

As I remarked at the beginning, the yeast disease of *Daphnia* is of special interest in so far as it helps us to understand the pathological processes of the higher animals. It strengthens the

statement that the white blood corpuscles and other phagocytes of vertebrates eat disease producers, and particularly the schizomycetes, and in this way are of considerable service to the organism. Although this conclusion had been drawn from the sum total of our knowledge of this subject, there has not been one conclusive example of the whole process of ingestion and digestion of fungus cells by phagocytes. Therefore we can criticize the conclusions which have been drawn concerning the presence of bacteria in whole blood cells. For example, R. Koch concluded, from his observations of various quantities of septicemia bacteria in the white cells of mice, that the bacteria could "penetrate the white blood cells and reproduce there." The process of penetration and reproduction could not be directly observed by him. . . . It seems to me more probable, that in this case also, the parasites were eaten by the blood cells. . . .

Because of the paucity of knowledge of this subject, it can be concluded that the pathological results obtained through studies on lower animals can be viewed as a new support for certain basic ideas of cellular pathology.

Comment

This paper shows how observations on lower animals can be of value in developing concepts of medical importance. There had been a long controversy, which still continues to some extent, as to whether the tissue or fluid elements of the body were responsible for natural resistance to disease. As we know today, both are important, but in Metschnikoff's time, there was no certain knowledge. As he mentions, bacterial cells had been seen inside white cells in the body, but no one had seen how they got inside. Did

they force their way in as invaders? If so, then the white cells were not defensive cells, but merely favored host cells of the parasite. Were the bacteria actively eaten by the white cells? If so, then the white cells might be a line of defense of the animal body.

In studying a fungus disease in *Daphnia*, Metschnikoff had an ideal system to examine this question. The animal is simple and transparent, and the fungus is large and easily seen without staining. As he describes, he could watch the

whole process in some detail. This work gave considerable encouragement to those who felt that the tissues of the body acted in disease resistance, and it was later possible to watch phagocytes of vertebrates ingest bacteria and verify that a similar process took place in higher

animals.

Remember that there is no mention here of artificial immunity. Phagocytosis may be viewed as a mechanism of resistance in both immunized and nonimmunized animals, but it is only the latter that Metschnikoff studied in *Daphnia*.