PLASMA CELLS.—In 1891 Unna described the elements which he called "plasma cells," believing them to correspond with certain granular connective-tissue cells to which Waldeyer had previously given the same name. Later studies indicated that the two were not identical, and Waldeyer advised the restriction of the term "plasma cell" to that of Unna, finding that the structures named by himself (Waldeyer) corresponded, at least in large part, with the "mast cells" of Ehrlich. Various papers on plasma cells have appeared since Unna's earlier publications, while their nature and properties have been the subject of numerous discussions, often lengthy and occasionally acrimonious. From this it appears that our knowledge of them is still far from definite. There is even some difference of opinion as to what a plasma cell is in fact. It has received its name on account of having *protoplasm* that may be stained by basic aniline dyes. This property is by no means peculiar to it, however, and is shared by several other varieties of cells; for instance, ganglion cells, lymphocytes, osteoblasts, certain giant cells, and mast cells. (In the case of mast cells, large granules contained in the body of the cell are the part stained, and they assume a different shade of color from that of the pure stain itself—metachromism.)

MORPHOLOGY.—With Unna's polychrome methylene blue (described hereafter) the protoplasm of the plasma cells is stained blue-violet, while the nuclei become blue. The outer part of the protoplasm stains more deeply than the inner part, leaving a pale zone around the nucleus. The nucleus is round or oval, and is usually placed eccentrically. Five to eight deeply stained masses of chromatin occur in the nucleus, chiefly around its border. Some observers have described a nucleolus. Two or more nuclei are occasionally present. In size the plasma cells vary from being of the dimensions of a leucocyte to objects of a much larger size—average diameters, 6 to 7 μ by 8 to 10 μ. In shape they are round, oval, roughly cubical, or elongated, according to whether or not they are confined by connective-tissue fibres or by the pressure of other cells. Evidences of both mitotic and amitotic division have been witnessed in plasma cells.

The above account is, in all essential respects, in accord with the views of von Marschalkó, which have been adopted by the majority of workers. It differs somewhat from the one originally given by Unna. According to Unna, the plasma cells have protoplasm that contains numerous granules capable of being stained. Such granules have not been seen by most other observers. It is not uncommon, however, to find plasma cells whose protoplasm is not homogeneous, but which contain small clumps and particles that stain unequally and irregularly. Cells having the metachromatic granules characteristic of mast cells, but otherwise like ordinary plasma cells, have been seen—plasma mast cells. These are unusual. Furthermore, according to Unna, the nuclei of plasma cells give up their stain much more easily than does the protoplasm, and in a preparation properly made the nuclei appear as unstained spots. Some have even maintained that two kinds of plasma cells exist, those of Unna and those of von Marschalkó. However, it is certain that the staining of the nucleus depends much on the technique employed.

OCCURRENCE.—Although they were at first supposed to belong only to pathological conditions, plasma cells have been reported as occurring in lymph nodes, in the lymphoid tissues of the spleen, and in the bone marrow, both in man and in the lower animals, in ligaments, in the framework of mucous glands of the tongue, and in the mucosa of the stomach and intestine in man. Information as to their distribution in normal tissues is not very full or exact.

On the other hand, so much has been written on their occurrence in diseased conditions that a mere enumeration of these conditions is impracticable. Unna's first accounts of plasma cells were based on sections of lupus. He directed attention to tumor-like collections of these cells. Such a collection he called a "plasmoma." It has since been learned that plasma cells are abundant in the lesions of tuberculosis wherever situated, in those of syphilis, leprosy, actinomycosis, and rhinoscleroma—*i.e.*, the so-called "infectious granulomata." They are also frequently seen in the stroma of carcinoma. In the main they are characteristic of rather chronic processes, where they constitute an important part of what is often termed round-cell infiltration. They are said to collect especially around small arteries. Their relations with the epithelioid cells of granulation tissue are in dispute. In acute inflammatory conditions, and especially in acute suppuration, they appear in smaller numbers. Plasma cells have, however, been demonstrated in acute inflammatory lesions, bacterial or otherwise, in keratitis in the rabbit, in recent granulating wounds in man and in the dog, in the lesions of typhoid fever, in the cell infiltration of acute interstitial nephritis, and in the stroma of the lung and even in the exudate late in lobar pneumonia. Further work is needed on their relations to the cells of sarcomata, and it may possibly yield information of use in diagnosis. The study of the plasma cells in diseases of the skin has been carried on by Unna with great energy; the results may be found in his "Histopathology of the Skin."

ORIGIN AND FUNCTIONS.—Unna held that the plasma cells were derived from connective-tissue cells, stating that he was able to detect all the necessary transitional forms between the two. This theory has obtained a few adherents. Most observers, however, believe that plasma cells are derived from the lymphocytes, and chiefly from the small lymphocytes. It is claimed by some, furthermore, that plasma cells may become connective-tissue cells and thus aid in the formation of fibrous tissue. This latter hypothesis would make the production of connective tissue from lymphocytes possible under certain circumstances, the plasma cells being an intermediate stage. It would modify existing ideas considerably and convincing proofs will be demanded before it can be accepted.

Some writers take a middle ground, believing that the plasma cells come in part from lymphocytes and in part from connective-tissue cells.

The functions of plasma cells in other respects are equally uncertain. It is to be noted that they are not distinctive of any particular disease or class of diseases. Their relative absence in acute suppuration is remarkable. According to Councilman and Mallory, they have the power of amœboid movement, they may occur inside the blood-vessels, and may be seen in the act of emigrating from the blood-vessels. It is doubtful if they possess phagocytic properties, and if so these are probably not energetic.

Other ideas that have been proposed as to their functions are purely speculative. It has been suggested that their peculiar staining property is the expression of diminished activity or degenerative changes, that it indicates an increase of activity, that it is due to their having taken up chromatin from other and degenerated cells, that they have some protective function, and that they serve to eliminate some unknown substance.

TECHNIQUE.—Fixation of tissues may be secured with alcohol, corrosive sublimate, Zenker's fluid, formaldehyde, or Müller-formol. Either paraffin or celloidin embedding may be used. Various methods for staining plasma cells have been proposed. Unna's alkaline or polychrome methylene blue gives satisfactory results; thionin or toluidin blue serves equally well. The plasma cells may sometimes be stained with hæmatoxylin. The following formula, which is one of many given by Unna, will be found serviceable: Methylene blue, 1 part; potassium carbonate, 1 part; distilled water, 100 parts. The solution must stand for periods varying from weeks to months before it is fit for use.

Stain in the methylene-blue solution, which may or may not be diluted, fifteen minutes or longer. The sections will be overstained.

Rinse in water.

Decolorize in water to which a few drops of "glycerin-ether" have been added, for a quarter of a minute or several minutes, as required, till differentiation of the structure begins to appear (one-per-cent. acetic acid, or alcohol alone serves nearly as well).

Rinse in water.

Complete the decolorization with alcohol.

Clear in oil of bergamot or xylol.

By this process the plasma cells are stained blue-violet, their nuclei and other nuclei and bacteria blue, the granules of mast cells violet to red. Epithelial cells take the blue stain, sometimes intensely, especially the horny layers of the epidermis; giant cells are frequently stained as well; also the products of certain degenerations, as amyloid and mucoid, which may show varying degrees of metachromism. *Herbert U. Williams.*

The literature of this subject up to June, 1900, will be found in an article by the present writer in the American Journal of the Medical Sciences of the same date. The most important articles that have since appeared are the following:
Almkvist: Arch. f. Dermatol. and Syph., Bd. lviii., 1901.
Askanazy: Centralbl. f. allg. Path., etc., Bd. xiii., 1902.
Beattie: Journ. Pathol. and Bacteriol., vol. viii., p. 129.
Bosellini: Soc. Med. Chir., Bologna, December, 1901. Abs. Centralbl. f. allg. Path., etc., Bd. xiii., 1902, p. 331.
Enderlen and Justi: Deutsch. Zeitschr. f. Chir., Bd. lxii., 1901.
Friedländer: Arch. f. klin. Chirurg., Bd. lxvii., 1902.
Harris: Journ. Amer. Med. Assn., vol. xxxviii., 1902, p. 634.
Herbert: Journ. Pathol. and Bacteriol., vol. vii., p. 91.
Mallory: Journ. Exper. Med., vol. v., p. 1.
Pappenheim: Virchow's Arch., Bd. clxv., 1901, clxvi., 1901, clxix., 1902.
Schlesinger: Virchow's Arch., Bd. clxix., 1902.

PLASMODIUM MALARIÆ.—The protozoan parasite which Laveran discovered in 1880 was designated by Marchiafava and Celli Plasmodium malariæ. The organism which is classified with the sporozoa has little resemblance to the multinucleated amœboid bodies to which the term plasmodium has been given by zoologists; but even though unsuitable the binomial name, in virtue of its priority, is applicable to the species to which it was originally given. Observations inaugurated by Golgi have shown the existence of three readily distinguishable varieties of parasite, each of which is capable of producing malarial fever. The organism discovered by Laveran and later studied by Marchiafava and Celli is the æstivo-autumnal parasite which causes the most severe type of intermittent fever, characterized by irregular periodicity and occurring most commonly during the late summer and fall. That type of malarial fever which recurs at regular intervals of two days is caused by a closely related parasite which, nevertheless, presents such well-marked peculiarities that it is usually regarded as a distinct species. A third parasite causes the quartan type of malarial fever, distinguished by paroxysms recurring at intervals of three days. If, in accordance with the opinion of a few observers, it should be shown that the three parasites associated with these various types of fever are varieties of a single species, the name Plasmodium malariæ is applicable to this variable organism. In the present article, however, the organisms associated with tertian, quartan, and æstivo-autumnal malaria will be described as separate species.

The parasites of the malarial fevers multiply within the red blood corpuscles of their human host. Recent research has demonstrated that they are capable of development within the body of certain suctorial insects, which have fed upon the blood of an individual suffering with malarial fever. A second individual is infected by the bite of such an insect, which thus acts as an intermediary host.

Geographical Distribution of the Malarial Parasite.—The general and local conditions which favor the occurrence and spread of malaria are such as conduce to the extracorporeal existence of the parasite. The presence of mosquitos capable of transmitting the organism is essential to the endemic occurrence of the disease, so that the geographical distribution of malaria is in great part dependent upon factors which aid the multiplication of certain species of mosquitos.

In Africa, malaria prevails in its most pernicious form on the west coast, especially in the neighborhood of the Congo and Niger rivers. South of the Congo malarial fevers become infrequent and disappear in the most southern part of the continent. Upper Egypt is free from the disease, which occurs with great severity elsewhere upon the Mediterranean coast.

In Asia malaria is prevalent upon the coast of Asia Minor, Arabia, and near the Persian Gulf. Endemic malaria abounds not only in the basins of the Indus and of the Ganges, but upon the tableland of the Deccan. In many of the East India islands severe malaria prevails, but in the Philippine Islands, though the disease is widely distributed, it is not particularly severe. Pernicious malaria occurs in places near the coast and along the rivers of China, but in Japan the disease is mild and infrequent. Of interest is the almost complete immunity enjoyed by Australia, New Zealand, and the islands of the Pacific.

In Europe malaria prevails in the southern part of Russia, particularly upon the shores of the Black and of the Caspian seas, along the shores of the Danube, and upon the peninsulas bordering the Mediterranean Sea. In Italy well-known seats of endemic infection are the plains and marshes of the western coast, including the Roman Campagna and the Pontine marshes. Here the disease is so prevalent that it has been designated Roman fever, and its frequency and severity in Italy have stimulated much of the investigation which, since the discovery of the malarial parasites, has explained the complicated life history of these organisms. Northern Europe, including the British Isles, is in great part free from malaria, though there is evidence that at an earlier period, particularly in England and in Denmark, it has occurred with severity in regions where it is now almost wholly absent.

In the West Indies, along the northern and eastern coasts of tropical South America, and in Central America malaria exists in its worst form. The disease is common in the southern part of the United States near the Gulf of Mexico and along the Mississippi and its tributaries. It occurs near the Atlantic coast with gradually diminished severity as far north as New York. Elsewhere are a few scattered localities where the disease is of mild type.

Malaria is pre-eminently a disease of tropical and subtropical countries, but prevails with diminished severity in many parts of the temperate zone. It is endemic in certain localities, particularly near the mouths and along the banks of rivers. In such localities are found the pernicious types of fever caused by the æstivo-autumnal parasite, while where the disease is less prevalent milder types, the regularly intermittent tertian and quartan fevers, are more common.

Conditions which Favor the Occurrence of Malaria.—The influence of temperature upon the occurrence of the malarial fevers is well illustrated by the preceding account of its distribution. In the endemic foci of the tropical and subtropical countries where malaria occurs in its severest form the disease prevails throughout the year. In the temperate zone as the poles are approached its frequency and severity progressively diminish with the temperature, and, according to Hirsch, malarial fever does not occur in localities where the mean summer temperature is below 15° or 16° C. Even in tropical countries its prevalence increases during the summer and reaches a maximum about the beginning of autumn. In temperate regions the disease may be limited to the warmer months of the year. The incidence of the disease in Baltimore, as described by Thayer, illustrates this condition. During January and February malarial fevers are almost absent, but the gradually increasing number of cases which occur during the spring and early summer are of the milder tertian and quartan type. Double tertian and triple quartan infections occur later in the summer, and the æstivo-autumnal parasite makes its appearance. Cases of æstivo-autumnal infection now increasing in number form a very large proportion of those which occur during September and October, and then, gradually diminishing in number, finally disappear completely during the winter.

Conformation of the soil and its saturation with water have an importance little inferior to that of heat in determining the local incidence of malaria. Standing water associated with abundant vegetation is always suggestive of a malarious region, and the name paludal or marsh fever illustrates the well-recognized relationship between the disease and marshy regions. The geological character of the soil does not influence directly the occurrence of malaria, but is of importance only in so far as it determines the saturation of the ground. Hence a clay soil is favorable to the disease, while a more porous, chalky, or sandy soil is less able to retain water; an impervious subsoil is especially capable of fulfilling this condition.

Since saturation of the soil has such an important influence upon the existence of the disease, the most malarious regions are the low-lying coasts and the marshy banks of rivers and lakes. Land which is submerged during a part of the year is believed to be particularly subject to endemic infection. The amount of rainfall has an important influence, and in tropical regions the disease usually reaches a maximum about the end of the rainy season.

Altitude, with its influence upon drainage and upon temperature, affects the local occurrence of malaria so that the severity of the disease tends to diminish as one ascends above the sea level. Nevertheless, severe malaria may be endemic upon high plateaus and even in mountainous regions, but here, according to Hirsch, it is the basin-like depressions that are most markedly infected.

Cultivation and drainage of malarial regions have in numberless instances been followed by the disappearance of the disease, but occasionally an opposite result is produced and endemics of severe malaria have followed the clearing of such lands. Moreover, outbreaks or exacerbations of the disease have followed extensive excavations of soil in making canals, railroads, and fortifications, but careful examination of the attendant circumstances have not infrequently shown that such disturbances have in various ways interfered with surface drainage.

Recent observations having shown that the malarial parasites pass a part of their life within the body of the mosquito, it has become obvious that the previously described conditions under which the disease occurs are such as favor the multiplication of these insects. Since the development of the larval mosquito occurs in stagnant water, poorly drained lowlands and marshes afford most suitable conditions, and heat and abundant atmospheric moisture are favoring circumstances. No malarious region has been found to be free from these insects, but on the other hand it is not surprising that malaria does not always occur where mosquitos abound; for in the first place, as will be pointed out later, only mosquitos of the genus Anopheles have been found to transmit the disease; and in the second place, the malarial parasite may not have found its way into a locality where mosquitos of this genus prevail. A correspondence exists between the habits of the mosquito and the seasonal incidence of the disease in temperate climates. With the beginning of winter many mosquitos are killed while a few hibernate. In the spring those that survive lay their eggs upon stagnant ponds and continue to multiply during the warm season. Marchiafava and Bignami state that during the fall mosquitos, many of which are infected, seek shelter within the houses, thus explaining the frequency of infection at this time and the occasional occurrence of house epidemics.

Even should mosquitos of the genus Anopheles and malarial parasites coexist in the same locality, external conditions will determine the prevalence of the disease. Investigations of the Italian observers have shown that the parasites within the mosquito develop best at a temperature between 20° and 30° C., but at 14° to 15° C. development does not occur. This fact accords with the observation previously mentioned, that malarial fever does not occur as one passes from the equator beyond an isotherm at which the mean summer temperature is below 15° to 16° C. (59° to 60.8° Fahr.).

Method by which the Malarial Parasite Enters the Body.—Before the discovery of the malarial parasite, two theories explaining the mode of malarial infection were much discussed. It was believed that the contagium causing the disease entered by one of two possible paths; either (1) by the digestive tract, being ingested with water, in which the infectious agent was thought to have its natural habitat; or (2) by the respiratory tract, being breathed in with the air.

Clinical evidence has been adduced to show that the disease is a water-borne infection. Supporters of this theory cited instances in which, of two neighboring communities, each using a different water supply, one was subject to malarial fever while the other escaped. It was believed that boiling of drinking-water was an efficient prophylactic measure. Such claims are found to have been based upon insufficient evidence, and in individual instances the difficulties of diagnosis between typhoid and malarial fevers were not clearly recognized. In recent years Italian observers have attempted to infect individuals with drinking-water obtained from localities well known to be malarious. Celli failed to infect individuals in Rome by the repeated administration of water brought from the Pontine marshes, and Zeri obtained similar results in a considerable number of experiments in which water from malarious regions was administered in large quantity to healthy subjects.

Certain clinical facts favor the alternate theory of air infection; for example, the well-known danger of infection after exposure to the night air in a malarious district may be cited. The contagium was believed to have its home in the water and in the soil of marshy malarious districts, whence it found its way into the air and was inspired by those exposed. Since the discovery of the malarial parasite numerous efforts to discover some phase of these micro-organisms in water or in the soil have proved futile, while the hypothetical means by which such bodies might reach the overlying air was difficult to conceive. Moreover, certain well-known facts are incompatible with this theory; for example, prevailing winds have little influence upon the spread of infection from an endemic focus, and the fact is frequently cited that upon vessels anchored off a malarial coast only those individuals become infected whose duties carry them ashore. This theory of air infection, unsupported by any convincing evidence, is now abandoned, since accumulating evidence has demonstrated that the malarial parasite is transferred from one individual to another by a suctorial insect, within which the micro-organism passes one stage in its life history.

Manson, in 1878, showed that Filaria bancrofti is carried from one human host to a second by the mosquito; Theobald Smith has shown that Texas fever of cattle is inoculated by a species of tic. These important discoveries have pointed the way to a clear recognition of the method by which the malarial parasites enter the human body and have helped to explain its life history outside. The solution of these difficult problems has received much aid from the studies of parasites which, occurring within the red blood corpuscles of birds, are so closely related to the malarial parasites that by some observers they have been regarded, doubtless erroneously, as identical species.

The possibility that malaria might be transmitted by the mosquito had been suggested long before the malarial parasites were discovered and the fact had been recognized that localities and conditions favorable to the disease are such as further the multiplication of this insect. Manson, in 1896, brought this theory into prominence. Bignami subsequently published a considerable amount of evidence to show that malarial infection occurs under conditions which favor the attack of mosquitos. When the wind blows on the Roman Campagna, he says, mosquitos hide close to the ground or beneath the trees and are transported little if at all from the locality in which

their larvæ develop. When the wind goes down at sunset, the insects rise in great numbers and attack men and beasts. The well-recognized danger of infection during the night is due to the nocturnal habits of the mosquito and is increased by sleeping in the open air. Many of the precautions which are taken by the inhabitants of malarious districts to ward off the fever are such as protect them from the attacks of insects. On the Pontine marshes relative protection is afforded by elevating dwellings on platforms six or eight feet high, for the insect tends to fly close to the ground.

Ross, working in India, has shown that the parasite of birds which is closely related to the malarial organism undergoes a series of developmental changes within the body of the mosquito, and subsequently found that mosquitos which had bitten infected birds were capable of transmitting the parasite to others which repeated examination had proved to be healthy.

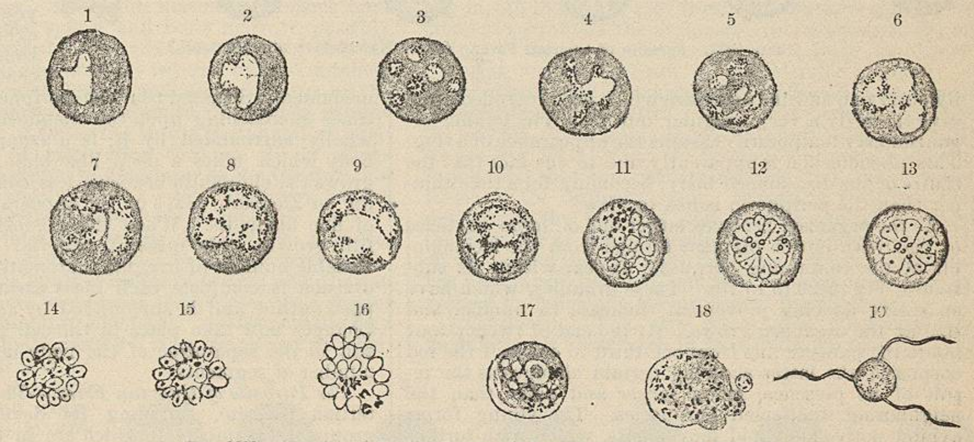


FIG. 3820.—Parasite of Tertian Fever. (After Marchiafava and Bignami.)

Grassi, in 1898, undertook a laborious investigation of the species of mosquitos found in malarious regions of Italy, and came to the conclusion that certain species are constantly present in districts of endemic infection. Where malaria prevails Anopheles claviger and other species of the genus Anopheles are abundant, while in non-malarial regions, though species of the genus Culex abound, he found the genus Anopheles unrepresented.

Direct proof of the agency of suctorial insects was finally brought by experiments of other Italian observers. Bignami brought to Rome mosquitos belonging to the genera Culex and Anopheles, obtained from a marshy region characterized by the severity of its malarial fever. An individual who had never suffered with malaria slept in the room in which these insects were liberated, and was exposed to their attack during more than a month. At the end of this time he became ill, suffered with chills and fever, and in his blood was found the parasite of æstivo-autumnal fever. In three subsequent experiments performed upon individuals who had never suffered with malaria, fever caused by the tertian or æstivo-autumnal parasite was produced by the sting of Anopheles claviger obtained from malarious localities; in every case recovery followed the administration of quinine.

In one of these experiments, insects, obtained, to be sure, from a malarious region, were allowed to sting a patient suffering with æstivo-autumnal fever. Subsequent examination of some of these mosquitos showed those developmental phases of the parasite which will be described later. Three such mosquitos, of which two were later found to be infected, were allowed to sting a healthy man. After an incubation period of from nine to twelve days, uncertainty being due to repeated exposure, fever of an irregular type ensued and the æstivo-autumnal parasite was demonstrated in the blood of the infected individual. It has been urged that these experiments were conducted in Rome, where malarial fevers are not uncommon. In order to meet this objection, Bignami sent to England where malaria rarely occurs, mosquitos infected with the parasite of tertian fever. P. Manson, Jr., who exposed himself to the sting of these insects, suffered a mild attack of tertian fever.

Demonstration that the parasites of malarial fever and the related organisms of birds undergo a peculiar process of development within the body of the mosquito, amplifies and confirms the experimental results just cited.

Parasites of the Malarial Fevers.—The micro-organisms which cause malarial fevers belong to the group of sporozoa known as Hæmosporidia. Recent observers have shown that they undergo two cycles of development: (1) One occurs within the human body where the organism, developing within the red blood corpuscles and multiplying by an asexual process, causes malarial fever; (2) parasites ingested by the mosquito when it attacks an individual suffering with the disease undergo a second cycle of development in the body of the insect. This second stage in the life history of the organism is preceded by a process of fertilization which occurs in the stomach of the insect and terminates by division of the parasite into a great number of reproductive bodies.

Golgi showed that the parasite which causes regularly intermittent fever of the quartan type differs morphologically from that associated with tertian fever. Both the tertian and the quartan organism within the human body develops in great groups, all members of which are at any given time in the same phase of development. The parasite of quartan fever completes its cycle in seventy-two hours, while the parasite of tertian fever repeats its cycle every forty-eight hours. It was subsequently shown that the organism present in those severer forms of malarial fever which prevail in the late summer and in the autumn months have distinctive peculiarities. In harmony with the irregularly intermittent course of the fever the parasite does not present the regular periodicity which characterizes the development of the other two varieties. Some observers have claimed that even among the parasites producing the irregular or æstivo-autumnal type of fever two varieties may be distinguished, but the distinctions which they emphasize are not sufficiently marked to establish the existence of more than one æstivo-autumnal parasite. Most observers recognize the existence of three distinct species of parasite; not only are differences observable within the human body, but during their development within the mosquito as well, morphological characteristics serve to identify the three varieties.

For the purpose of clinical diagnosis the malarial parasite is best observed in thin films of freshly drawn blood. Much attention has been given to the study of the parasite fixed and stained by a variety of methods, in great part modifications of that used by Romanowsky, who employed a mixture of eosin and methylene blue. The body of the organism stains blue, while its chromatin substance takes a lilac color. Wright has recently so modified this method that it may be used for the purpose of clinical diagnosis.

The Parasite of Tertian Fever.—The earliest phase of the parasite, Fig. 3820 (1 and 2), found within the red blood cell is represented by a minute clear body, often circular in outline, with a diameter about one-fourth that of the corpuscle. Active amœboid movements are readily observed, and the processes which are protruded often give the body a very irregular outline. The hyaline organism may temporarily assume the appearance of a ring. This phenomenon is apparently due to the fact that the centre of the disc-shaped body, becoming for a time thinner than the periphery, is lost to view.

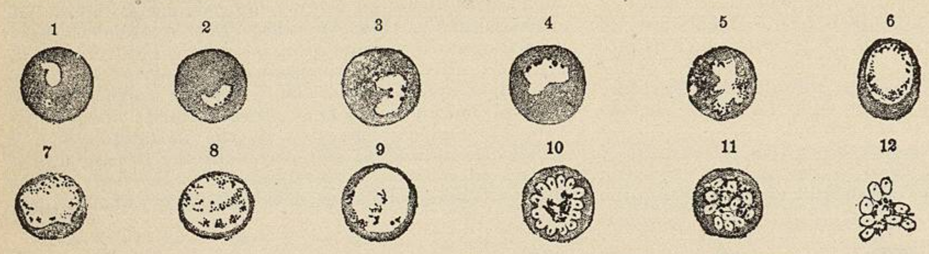


FIG. 3821.—Parasite of Quartan Fever. (After Marchiafava and Bignami.)

As the organism becomes larger one or more particles of yellowish-brown pigment formed from the hæmoglobin of the containing corpuscle appear within its substance, Fig. 3820 (3 to 10). These granules, which have an active dancing movement, increase in number and size as the organism grows. At the end of twenty-four hours the parasite fills from one-third to one-half the red corpuscle, the latter showing certain changes as the result of its presence, being larger and paler than the neighboring unaffected corpuscles. The young forms exhibit active amœboid movements, which with further growth become less conspicuous. At the end of about forty-eight hours the organism has reached its full size, about that of a normal red corpuscle, and is ready to undergo the process of segmentation. The parasite, Fig. 3820 (12 and 13), having assumed a circular outline, the pigment granules collect into a clump, often a solid block, which usually lies in the centre of the body. The organism acquires a dense opaque appearance not observable in the amœboid stage. Refractive dots may be seen near the periphery, occasionally in the more central portion, and radial striations extending inward from corresponding indentations at the margin appear between the refractive points. The striations represent lines of separation which divide the body into segments corresponding in number with the refractive dots; very frequently there is formed a peripheral circle of segments, within which, nearer to the central pigment, is a second group. These bodies, numbering twelve to twenty or even thirty, assume a circular outline, the aggregation being still surrounded by a pale, very inconspicuous yellowish rim, the remains of the much-enlarged and decolorized corpuscle. Fig. 3820 (11). Finally, this ruptures and sets the bodies free in the plasma. Fig. 3820 (14 to 16).

As previously mentioned, all the members of a group segment at approximately the same time. During a period of about three hours before the chill, the temperature rises gradually and segmenting bodies can be found in the blood. During the chill and with the beginning of the hot stage, they are present in greatest number. It seems probable that a toxic material is set free when the parasite segments and that this toxin causes a febrile reaction. Occasionally two groups of parasites are present in the same blood and double tertian fever results. The two groups undergo segmentation on alternate days, and at any given time parasites in two phases of development are discoverable in the blood.

In addition to the full-grown parasites which undergo segmentation are even larger bodies situated within enlarged pale corpuscles. Since they do not divide to form reproductive segments, they were at one time regarded, in part at least, as degenerate forms. Some of them exhibit the remarkable phenomenon known as flagellation, Fig. 3820 (19), and their significance will be subsequently explained in describing this process.

Certain details of internal structure not observable in the living organism may be studied in stained specimens. In preparations treated with eosin and methylene blue according to the method of Romanowsky, the existence of nuclear material may be demonstrated. In such specimens young hyaline forms exhibit an external zone of blue color, the central part remaining unstained. Situated near the periphery of the body, at times encroaching upon the unstained area, more rarely wholly surrounded by it, is a compact round or oval body which takes a deep lilac stain. As the parasite grows the chromatin becomes less compact and, according to Ziemann, takes on the appearance of a collection of fine filaments. When the organism has attained its full growth, this mass of material divides to form a variable number of irregular chromatin bodies, and when division is complete each mass assumes a round compact outline and is surrounded by an achromatic zone. Changes now take place in the cell protoplasm and result in the separation of the body into a corresponding number of segments.

The Parasite of Quartan Fever.—The quartan, like the tertian parasite, pursuing its development in great groups, all members of which are in the same phase at any given time, repeats its cycle in seventy-two hours. As with the tertian organism, the malarial paroxysm is simultaneous with the process of segmentation, and, when the result of an infection with a single group, occurs every fourth day. When more than one group are present, they reach maturity on different days, the members of two groups never undergoing segmentation on the same day. When the blood is infected with three groups paroxysms occur daily.

The quartan parasite resembles closely the organism of tertian fever; nevertheless, certain differential peculiarities can be noted. The amœboid hyaline bodies Fig. 3821 (1 and 2) are indistinguishable from those of the tertian parasite, but with increase of size and the acquisition of pigment Fig. 3821 (3 to 9) the characteristic features become evident. The pigment of the quartan organism occurs in coarser granules having a deeper brown color. The body of the parasite has a more refractive appearance and its outline within the corpuscle is much more distinct; the amœboid movements are much less active, and as early as the second day they almost or completely cease. The changes produced in the containing red corpuscle differ from those caused by the tertian organism; the corpuscle is not swollen and decolorized, but becomes somewhat shrivelled and assumes a deeper color of a greenish copper-like hue. As the parasite increases in size the surrounding rim of the corpuscle becomes smaller, and at the beginning of segmentation, about ten hours before the paroxysm, though present, it is almost imperceptible. The pigment Fig. 3821 (10) collects toward the centre of the body, and in doing so usually assumes a radial arrangement not seen in the tertian organism. The segments are fewer in number, often not more than from six to ten, and are arranged regularly in a single row about the central pigment mass. In stained specimens the details of structure previously described for the tertian parasite are observed and the same division of the chromatin substance is found to precede segmentation.

The Parasite of Æstivo-autumnal Fever.—The severer forms of malarial infection, including those presenting pernicious symptoms, are caused by an organism which differs in several important particulars from those pre-

viously described. Since, as already mentioned, its development, unlike that of the tertian and quartan parasites, does not take place in great groups, of which the members mature at approximately the same time, its cycle cannot be followed with the same readiness. And in accordance with this irregularity of development the symptoms of the disease do not present the same periodically paroxysmal character observed in the other two types. Moreover, the parasite of the irregular fever does not undergo its whole development within the circulating blood, and all stages of growth cannot be observed in specimens obtained in the ordinary way. In the blood from the peripheral circulation only the youngest forms are found, the subsequent development taking place in the internal organs. The more mature organisms tend to accumulate in the spleen and bone marrow, possibly because, as suggested by Bastianelli and Bignami, the red corpuscles being profoundly injured by the contained parasite act as foreign bodies and are taken up by the cells of these organs. The absence of definite groups and the difficulty of following in the peripheral circulation the process of maturation through all its stages have made it impossible to determine as yet the duration of the cycle of development. Certain observers believe that it lasts two or three days, or even longer.

The youngest intracorpuscular form of the parasite, Fig. 3822 (1 to 7), is represented by an amœboid organism resembling the hyaline bodies of tertian and quartan fever; it is, however, somewhat smaller and has a greater tendency to assume a ring-like form. Such a parasite may be seen to assume alternately a ring shape and an amœboid form. With further growth pigment granules are acquired, and occasionally one sees in the blood from the peripheral circulation a small body, one-fifth the diameter of the red corpuscle, containing one or two minute granules of dark brown pigment. Larger pigmented forms are only very rarely found in the peripheral circulation, so that the later stages have been studied mainly in specimens of blood aspirated from the spleen. As the parasite grows the pigment increases in amount, but does not become so abundant as in the tertian and quartan organisms. Indeed, Marchiafava and Celli have described instances in which the parasite attained its full growth and underwent segmentation without any formation of pigment. The invaded corpuscle takes on a greenish brassy color, and wrinkling and crenation may be observed in corpuscles containing even the smallest hyaline bodies. The full-grown forms, Fig. 3822 (17 to 19), vary considerably in size, often exceeding in diameter one-half that of the containing red cell. The pigment collects into a clump, Fig. 3822 (Fig. 21 to 23), and is finally caked into a solid block, usually situated near the centre of the body. The organism acquires a refractive waxy appearance and divides, in a manner similar to that exhibited by the tertian organism, into from eight to sixteen segments, Fig. 3822 (24), which are finally set free in the plasma by the rupture of the containing rim of the corpuscle. In stained specimens the existence of chromatin resembling that of a tertian parasite can be demonstrated; it undergoes the same division and arrangement previous to segmentation.

In the blood of patients suffering with æstivo-autumnal malaria are found bodies not present with the regularly intermittent types—the crescents and ovoid bodies described by Laveran. The crescentic bodies, Fig. 3822 (36 to 38), present in the blood after the fever has lasted a week or more are longer than the red blood corpuscles, somewhat more than half their diameter across, and with very refractive protoplasm. A pale yellow rim projecting in bib-like form (38) from the concave side of the crescent surrounds the organism; this represents the remains of the corpuscle in which the body developed. Pigment, usually present in considerable quantity, is either distributed throughout or collected into a mass situated near the centre. The ovoid bodies, Fig. 3822 (34 and 35), differ from the crescents only in shape. Early stages, Fig. 3822 (28), in the formation of these bodies are found, and they represent transitions from the youngest forms of the ordinary cycle. Becoming fusiform, they develop in the red cells, and, as they exceed in length the diameter of the corpuscle, become bowed in order to accommodate themselves to its shape. Their significance has been much disputed, but, now understood, will be explained in describing the process of flagellation.

Flagellation.—The very remarkable phenomenon of flagellation is observable in freshly drawn blood. With tertian infection, within a week after onset of the disease, large spherical bodies, which do not segment, are present in the blood. The periphery of such a body in a specimen of fresh blood now unsurrounded by a rim of corpuscle is seen to undergo violent undulation, when suddenly there appear one or more thread-like filaments which lash about so actively that their outline is distin-

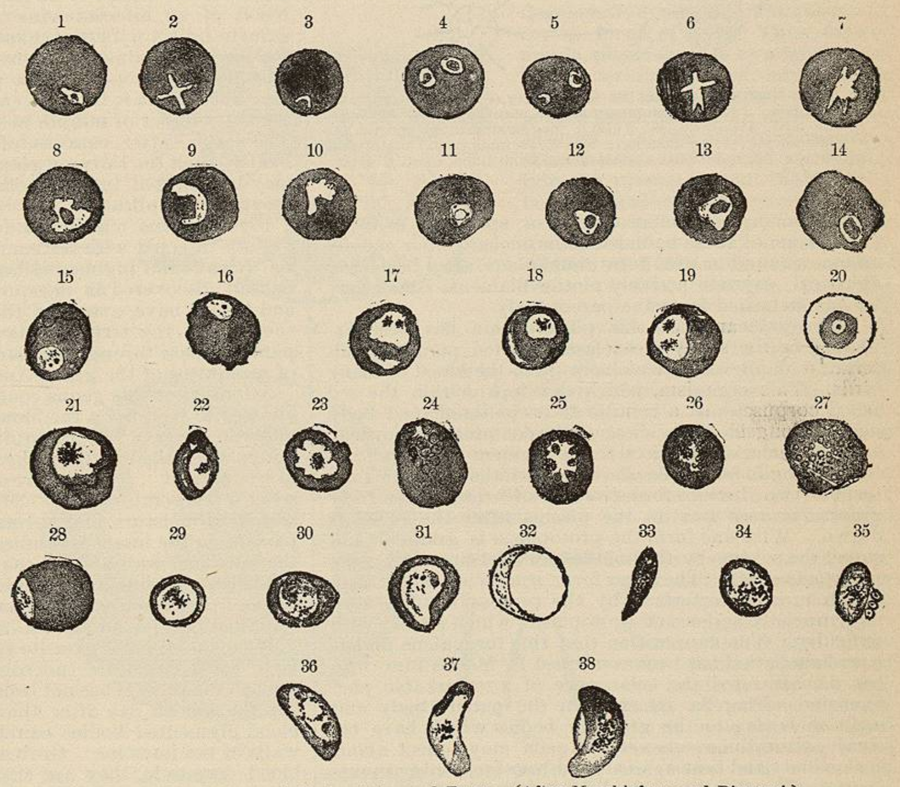


FIG. 3822.—Parasite of Æstivo-Autumnal Fever. (After Marchiafava and Bignami.)

guished with difficulty. Fig. 3820 (19). A flagellum with a club-like enlargement at one end not infrequently separates from the parent body and floats away in the plasma with an active undulatory motion. It has been shown that the flagella contain part of the chromatin substance. All the extracellular bodies observable in freshly drawn blood do not undergo flagellation.

With the quartan infection a similar process is observable. Flagellate bodies, however, are encountered with much less frequency than in cases of tertian fever.

In cases of æstivo-autumnal fever it is the crescents which undergo flagellation. In a specimen of fresh blood some of these bodies remain unchanged or merely assume a round or oval form; but others, after becoming spherical, extrude actively motile filaments which may become detached from the parent body.

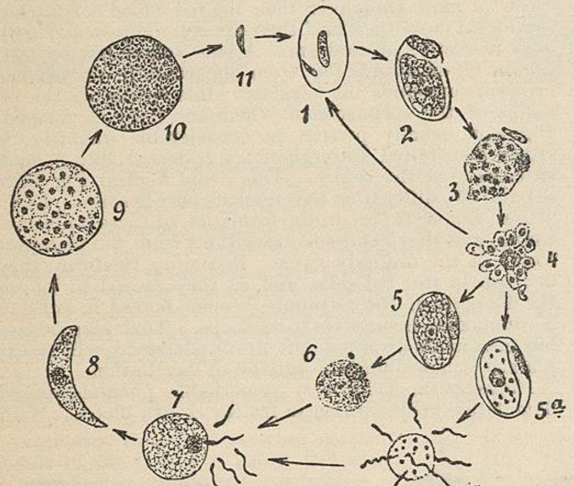


FIG. 3823.—Diagram Showing the Life History of the Avian Parasite, Proteosoma. 1 to 4, Development of the parasite within the blood of the bird; 4, merozoites; 5 and 6, macrogamete; *5a* and *6a*, microgametocyte; 7, fertilization; 8 to 10, formation and maturation of oöcysts; 11, sporozoite. (After Grassi from Lühe, *Cent. f. Bakt.*, 1900, xxvii.)

The significance of this phenomenon has been explained by the study of a closely related parasite, Halteridium danilewsky, which occurs in the blood of many birds. This organism, which develops within the red blood corpuscles as a semilunar or halter-shaped body curved alongside the nucleus of the containing corpuscle, like the malarial parasites forms pigment granules from the hæmoglobin. Opie showed that the parasite may assume two distinct forms, either of which when fully grown becomes free in the plasma after the blood is drawn. With one form the protoplasm is granular and stains deep blue by the method of Romanowsky; the nucleus is small. The other form, somewhat larger than the first, is characterized by the possession of a very large nucleus and scant protoplasm which stains with difficulty. The supposition that this form alone undergoes flagellation has been confirmed by MacCallum, who has demonstrated the occurrence of a remarkable phenomenon. Flagella break from the parent body and make their way to the granular bodies which have become extracellular. Several flagella may collect about such a body and beat against it with active lashing movements. One flagellum finally projects itself into the substance of the body, with which it becomes merged. The process is to be regarded as one of fertilization; the granular body with small nucleus is the female element, or, according to zoological nomenclature, the macrogamete; the flagellum is the male element, or microgamete, its parent body being designated by the term microgametocyte. The fertilized body remains quiescent for from fifteen to twenty minutes, when it assumes an elongated form and becomes capable of very active progressive movement, constituting the pseudo-vermiculus described by Danilewsky.

Flagella-formation observable with the three varieties of malarial parasite doubtless represents a similar process of fertilization, though only in the case of the æstivo-autumnal parasite has the phenomenon been actually observed. With both the tertian and the æstivo-autumnal forms morphological differences have been established between the microgametocyte from which arise flagella or microgametes and the macrogamete which undergoes fertilization. With the æstivo-autumnal parasite those crescents which do not flagellate, like the analogous macrogamete of the halteridium, stain more deeply and are granular. The formation of a body similar to the pseudo-vermiculus of birds has not been observed.

Life History of the Malarial Parasites in the Body of the Mosquito.—Study of the parasites in birds has here again pointed the way to an understanding of the life history of the malarial organisms outside the human body. Ross, working in India, directed his attention to the extracorporeal development of the avian parasite, Proteosoma grassi, because at the time cases of malarial fever were not available. Observations of Manson upon the development of filaria in the body of the mosquito suggested the agency of these insects. Ross proved that the mosquito can act as an intermediary host in transferring infection from one bird to another, and showed that the parasite develops within the body of the insect. The process of flagellation occurs with much activity in the middle intestine of an insect which has fed upon the blood of an infected bird. The flagellum or macrogamete unites with the microgamete, and the so-called pseudo-vermiculus which results, endowed with active motility, makes its way into the wall of the mosquito's intestine, where it becomes encapsulated and divides into a great number of minute bodies designated sporozoites. The latter, after rupture of the containing capsule, finally reach the salivary glands of the insect and hence may be injected into a second bird, there to undergo asexual multiplication.

In mosquitos which had fed upon the blood of individuals infected with tertian and æstivo-autumnal malaria, Ross found pigmented bodies similar to those which he had discovered in mosquitos. Bignami, Bastianelli, and Grassi have extended these observations and have shown that the tertian, quartan, and æstivo-autumnal parasites pass through similar changes within the body of mosquitos of the genus Anopheles.

An insect of this genus confined in glass test tubes is allowed to feed upon the blood of patients infected with malarial fever. The mosquito is so voracious that it stings when the mouth of the tube is applied to the skin of the patient. It is then kept confined in a larger vessel at a temperature of 20°–30° C., and is supplied with abundant moisture and vegetable matter for food. The parasite in the insect is studied after varying periods, in the intestinal wall and in the salivary glands, prepared by delicate teasing and examined in salt solution. Sections of the insect hardened and stained for microscopic examination give additional information.

With the tertian parasite fertilization of a macrogamete by a flagellum (microgamete) doubtless occurs, though the process has not been actually observed. During the second day after the insect has fed on malarial blood pigmented bodies can be found in the muscular walls of the intestine. Grown to twice the size of a red blood corpuscle, they are sharply outlined and possess homogeneous or vacuolated protoplasm. In stained specimens the chromatin substance is found to have increased in amount and may have undergone division into several small masses. Increasing in size, the parasite acquires a refractive capsule, and on the third day its contents have divided into a varying number of small bodies, each containing a part of the chromatin substance; between these lie the pigment and a small amount of undivided cytoplasm. This cyst-like body

increases in size and within it is formed an increasing number of small bodies. Finally, on the sixth day, the parasite, which has grown to such size that it projects into the body cavity of the insect (compare Fig. 3825 showing Proteosoma of birds), contains a great number of slender bodies with pointed extremities, sporozoites, Fig. 3825 (*C*) each containing a particle of nuclear substance demonstrable only in stained specimens; they are arranged in groups side by side. The containing capsule ruptures and the sporozoites are set free in the body cavity, whence they make their way to the salivary glands of the insect. Should a mosquito so infected sting a human being, parasites are injected with the irritant fluid secreted by the gland. Developing within the red blood corpuscles, the organism now begins in its new human host the asexual cycle of development with which is associated tertian malarial fever.

A few observations have shown that the quartan parasite passes through a series of phases corresponding to those just described, but when mosquitos are allowed to sting patients suffering with quartan fever, in only a small proportion of the experiments are developmental stages of the parasite obtained. The small number of flagellate forms observable in the blood of patients suffering with quartan infection may explain this fact as well as the relative infrequency of this type of malarial fever.

The development of the æstivo-autumnal parasite in the mosquito may be readily observed. On the seventh day after the mosquito has stung a patient infected with the disease, cyst-like bodies project into the body cavity of the insect and are filled with sporozoites, which, though more numerous, resemble those of the tertian parasite. Even before sporozoites are formed, the æstivo-autumnal parasite is recognizable by the character of its pigment and by the high refraction of its cytoplasm.

Terms in general use by zoologists have been introduced to designate various phases of the asexual generation of the malarial parasite in man and of its sexual generation in the intermediate host, the mosquito. Some of these have been mentioned. The microgamete or flagellum, derived from the microgametocyte or flagellate body, unites with the more granular macrogamete, and as the result an oöcyst is formed within the stomach wall of the mosquito. Division of cytoplasm preceded by nuclear division gives rise to a great number of nucleated bodies known as sporozoites. The latter, injected by the mosquito, are capable of transmitting malarial infection, since they are capable of development within the red blood corpuscles of their human host. Reaching a certain size, the intracorpuscular parasites divide without preceding fertilization into a variable number of bodies, which may be termed merozoites, each capable of re-entering a red blood corpuscle and undergoing the same process of multiplication. A certain number of merozoites, however, are not destined to multiply by such asexual division, but give rise to macrogamete or microgametocyte as already described. By union of the male and female elements within the stomach of the mosquito is formed a body which is capable of development in the intermediary host. An analogous alternation of asexual and sexual generation occurs with other protozoan micro-organisms, notably those belonging to the order Coccidia. Asexual reproduction is the means by which a few parasites which have gained entrance multiply in the new host. In the case of the malarial parasite, relatively few organisms injected by the mosquito multiply to form the great number characteristic of the malarial fevers.

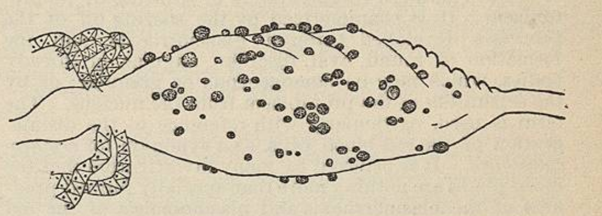
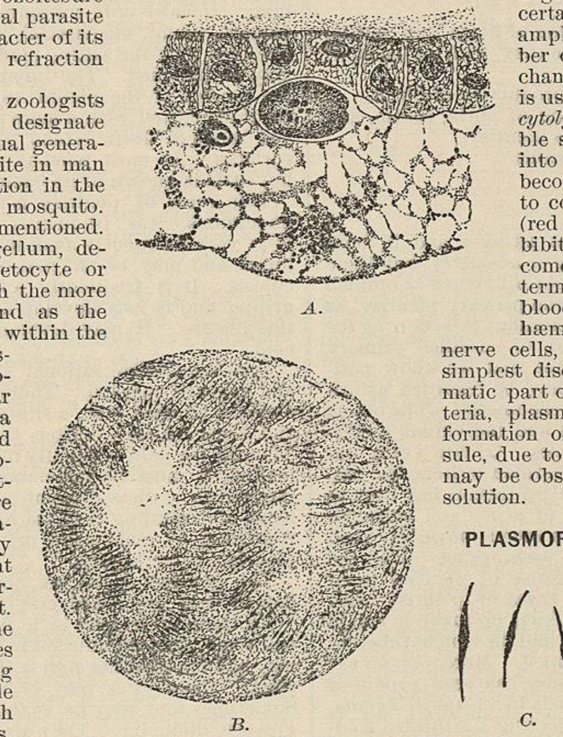


FIG. 3825.—Oöcysts of Proteosoma in the Wall of the Middle Intestine of the Mosquito. (After Ross from Lühe.)

Eugene L. Opie.


A. *B.* *C.*
FIG. 3824.—*A*, Parasite of Æstivo-autumnal Fever in the Wall of the Middle Intestine of Anopheles; *B*, formation of sporozoites within the oöcyst; *C*, ripe sporozoites. (After Grassi from Lühe.)

PLASMOLYSIS is the term used in a general sense to denote the disorganization of the achromatic part of the cell, in opposition to *karyolysis*, which is applied to the complete disorganization of the nucleus. *Achromatolysis* is, accordingly, used as a synonym for plasmolysis. The word plasmolysis, having been introduced by a number of investigators working along special lines, is frequently used in a more narrow sense to indicate the destruction or degeneration of the protoplasm of certain forms of cells. Thus, for example, plasmolysis is by a large number of writers applied only to such changes in the red blood cells, and is used interchangeably with *erythrocytolysis*. In this condition the soluble substances of the red cell escape into the plasma so that the red cells become smaller (microcytes) or come to consist only of the outer envelope (red cell shadows). Through the imbibition of fluids such cells may become swollen. Grawitz uses the term to indicate solution of the red blood cells and the production of hæmoglobinæmia. In the case of nerve cells, plasmolysis is applied to the simplest disorganizing changes in the achromatic part of the cell. In the case of bacteria, plasmolysis is used to indicate the formation of clear spaces beneath the capsule, due to the shrinking of the plasma, as may be observed in bacteria held in a salt solution. *Aldred Scott Warthin.*

PLASMORRHEXIS is the term applied to processes of disorganization in the protoplasm of the cell, in opposition to *karyorrhexis*, which is used to denote similar processes in the nucleus. By the majority of writers plasmorrhexis is applied to these changes as occurring in the red blood cells alone, and the word is used as a synonym for *erythrocytorrhexis*. The process is characterized by the formation of small granules or globules in the protoplasm of the red cell, and the escape of these from the cell; or the formation of minute prickles or globules over the surface of the cell, giving it an appearance of a gooseberry or mulberry; or the formation of variously shaped