

CLXXVII. From laboratory of clinical professor M. I. Afanasyev at Nikolaevsky Military Hospital.

## To a question of a structure of malaria parasites.

(Preliminary article.) D..L Romanowsky

The parasites causing malaria were assumed already from time immemorial, but are really described for the first time by *Laveran*. The subsequent supervision of many scientists (*Richard, Marchiafava, Celli, Golgi, Grassi, Canalis, Guarnieri, Autolosci, Angelini, Sternberg, Councilman, Osler, Swordsmen, Sakharov, Hentsinsky, Titov*, etc.) made in different places of the globe undoubtedly proved continuous presence of this parasite at blood of marsh fever patients and his role at production of marsh diseases and their consequences; at the same time also the circle of development of this parasite in connection with fever attacks was tracked. But still sorted parasite doesn't have still a strong place in classification, there is no certain name also. *Laveran* called it haematozoaire de paludisiue, Italians - plasmodium malariae, *W. Osler* - haematomonas malariae, *Swordsmen* - haematophyllum malariae, and recently *Grassi* and *Feletti* allocated two look - haemamoeba and Laverania.

It seems to me that the main reason of disagreements consists in insufficient knowledge of biology and morphology of a parasite. Difficulty of studying of the first is complicated by impossibility until recently to receive such nutrient medium in which it would be possible to breed a parasite and to observe it at desirable to us conditions. Still this parasite isn't found free in the nature, despite exact researches of water, the soil and air in marsh districts yet.

The researches of animals made by the prof. *Danilevsky* and *Shalashnikov*, especially the cold-blooded and the birds living in marsh districts showed that in blood of these animals parasites identical with meeting in blood of marsh fever patients, but quite often not having any noticeable adverse effect on the organism which sheltered them<sup>1</sup> come across). This comparative parasitology of blood helps studying of a dark question of biology of a malaria parasite.

Studying of morphology of the parasites who are found in blood of marsh fever patients too represents many difficulties which depend partly on the size of the studied object (frequent less than 1/10 red balls), and partly from its other properties.

At first saw in the one the nubbin of plasma (plasmodium) capable to the amoeboid movements without differentiation traces. As the last in general in live animal sections difficult differs, of course, in live and thus still so small organism as a marsh parasite, it is hardly possible to see a structure; on it the statement of *Celli* and *Guarnieri* that they saw nuclei in live parasites, can excite some doubt - especially as other authors, for example, *Sakharov*, says that it isn't possible to see nuclei at any magnification. And meanwhile in the proof of presence of a nucleus the solution of the most part of a dark question of morphology of a parasite as it is believed by so competent researchers as *Grassi* and *Feletti*. The latest facts, as well as theoretical reasons force to recognize behind a nucleus huge value and in morphological development of cells, and in their physiological activity (prof. *S. Zh. Lukyanov*); it is necessary to consider it on so many essential necessary accessory of any cell that it is rather possible to allow existence - a naked nucleus, than nuclear-free protoplasm. *Sacharias* believes that we don't see sometimes a nucleus,

can be only because it doesn't react on known to us stains now. From here naturally the question is, whether it is possible to allow at a present condition of our knowledge existence of nuclear-free organisms, which number, thanks to researches of modern naturalists, constantly decreases and to which it was necessary to attribute parasites of marsh diseases until recently. If we with a high probability can tell that there is no active cell without nucleus, naturally in a marsh parasite to look for a nucleus especially as it is proved at the majority of protozoa and even at identical, by *Danilevsky*, the parasites who are found in blood of birds, though *Shalashnikov*, describing cytozoon, says that «it is represented uniform homogeneous; more differentiated part, as. for example, nuclei, it wasn't possible to see yet, despite use of various dyes.»

The proof of presence of a nucleus, except scientific, so to speak, theoretical value, has also practical, diagnostic application, because in red balls can turn out. various, blue figures when staining by methylene blue and besides malaria, even in healthy blood, on as were specified by opponents of the doctrine about parasites of malaria and defenders of the last *Celli* and *Guarnieri* saw also which gave even the corresponding drawings. Of course, who is well already familiar with a marsh parasite, that will not allow such mixtures, but also the similar mistake is in practice possible; and therefore the finding for more exact distinctive recognition of a parasite convenient and thus has whenever possible practical way the basis and from this point of view.

The first work in this direction was performed by *Celli* and *Guarnieri* last year at what they investigated blood of patients with 4-day fever. In 1884 *Marchiafava* and *Celli*, staining with methylene blue parasites on dry preparations of blood, distinguished in them 2 parts: external, dark - to an ectoplazm, and internal, pale - to an endoplazm.

*Golgi* in sporulation forms, and thus only at 4-day fever, saw the lustrous little body in the center of a nubbin which was strongly stained which he recognized as a nucleus.

Last year *Celli* and *Guarnieri* after «vain attempt» to find out a structure of the examined parasite in all nowadays known ways of fixing and staining applied at last the *Bizzozero* method of blood staining in vivo, using for this purpose methylene blue solution (nonrotten prepared) in serumal liquid (belly dropsy). This way they received (in an ameoboid step of a parasite) an ectoplazma in which the pigment collects, and smaller but to volume the endoplazma which more weakly is stained, always pigment-free and located that in the center, on the periphery of a parasite; in this endoplazma also lies the nucleus surrounded with a light rim that is weak stained, with strong stained network. Too division on ecto- and to an endoplazma is noticed and in spores (a form of daisies), and in an endoplazma strongly stained point is visible.

*Sakharov*, investigating work of authors, believes that they were misled because in an endoplazma «at any magnifications it is impossible to notice nucleus, and everything forces to think that it simply the part of a blood ball taken by the met and merged plasmodium psevdopodiya.»

Apart from researches of the same authors evidential *Grassi* and *Feletti*, in turn, made researches in the same direction and «after many doubts came at last to the desirable decision.»

In essence they, "having expediently changed" the way *Celli* and *Guarnieri* found out and more clearly proved that predecessors saw them. Besides, they tracked a nucleus during its division. According to the description of authors, the vesicular, big, clear nucleus is similar to that

<sup>1</sup>) Article of the respected author is sent before publication of the last article of the prof. *Danilevsky* (see above. p. 1063). Editor.

at rhizopodes and is put in plasma in which there are strongly stained grains. The nucleus located the mostly eccentric has gentle often not clear membrane, nuclear juice and a nuclear network. Ecto- and endoplazma of *Grassi* and *Feletti* don't distinguish. The squeezed statement without drawings, and, above all absence of the description of the way («expedient modification») used by them is done by researches of authors not certainly convincing though *Grassi* has to be recognized as the great expert on the protozoon and though it already proved a nucleus at other protozoa.

In any case, even confirmation already found, in a type of importance of a question, will be useful, especially if the way of applied research is other. I also dare to state in brief the results received by me and my way of research that the colleges having more material could check and add my work.

Being engaged in a blood test of marsh patients (at 3-day fever), especially high-quality and quantitative changes of white balls in connection with presence of parasites, I couldn't use Ehrlich method (on dry preparations of blood) at which parasites are almost not stained, and was forced to look for a different way which would stain also nuclei of white balls and a parasite, and the nucleus assumed in it.

Needless to say that the *Bizzozero* method isn't applicable for my purposes as at it can't be any speeches about the mutual quantitative relation of blood corpuscle and the parasites. I won't speak about research of liquid blood and I will state only my way of research of dry preparations of blood which got out from a finger puncture with well-known precautions.

The blood applied on an cover glass with a thin layer (previously between two cover glass), is instantly fixed over a gas or spirit flame, and then for final fixing 45 - 60 minutes in a dry bath at 105 - 110 ° C. For staining the following mix found me is used, it is best of all freshly prepared: 2 volumes of the saturated filtered water solution of methylene blue and 5 volume of 1% eozin water solution (soluble in water).

In the graduated cylinder (10 sm<sup>3</sup>) I pour blue solution, add solution of an eozin, stir with a glass stick and pour out on an watch glass where I put to float a preparation; I cover an watch glass with other one, the last is necessary, especially at long leaving of a preparation in stain because water evaporates, moreover, due to the greater strength of the solution is obtained many known «metallic» plaque, very tightly adhering to the preparation. Obtained by mixing stain precipitate (filter must not) is not harmful, since it is easily detaches during the subsequent washing of the preparation in water. Good staining comes in 1 hour, even better in a day, but then it is required to wash out a preparation, of course, more long; for bigger distinctness it is good to rinse the last in the strong alcohol deleting excessive stain.

At warming up staining comes in 3 - 5 minutes, but thus it gives more a deposit, and a precipitate, and therefore preparations lose in clarity and beauty much; for this reason I also don't advise to heat, especially that who has still no skill in finding of a parasite. For the diagnostic purposes (at experience) it is possible not to heat blood in a dry bath, and directly to stain after fixing over a flame; then all research will take 20 - 30 minutes. I examine preparations directly in water with uniform immersion system, and for storing I mounts them in the Canadian balm with a xylol (1/3), and preparations don't become colorless.

On just described way I managed to receive such small forms of a parasite, which at other ways (*Titov, Hentsinsky*) - mainly, by the reason of weak staining - are difficult distinguishable

. which at other ways (*Titova, Hentsinsky*) - mainly, by the reason of weak staining - are difficult distinguishable; this difficult distinguishability in the youngest parasites is connected with small dimensions of protoplasm, and with weak staining of nucleus in methylene blue I believe.

In my preparations I obtain always the following picture: red balls are stained in pink color, protoplasm of eosinophils - in saturated-pink, a parasite of malaria and protoplasm of lymphocytes - in light blue, blood plates and nuclei of white balls in dark-violet, nuclei of parasites - in purple-violet, protoplasm of leukocytes in weak-violet at what it is possible to see transitional colors from light blue protoplasm of lymphocytes to violet leukocytes.

In each parasite, from hardly noticeable in a red ball to occupying all ball, always sharply distinguishable, violet nucleus surrounded with a colorless rim (halo, Hof), round, ovoid, quite often wrong form, is more often as if triangular that probably depends on group of a chromatin network is clearly visible. In adult parasites I managed to see the thinnest structure of a nucleus at what it represented an ovoid nimbus from the points and threads which are strongly stained, but not reaching the external periphery of a light rim. I didn't manage to see any cover of all nuclei though sometimes (especially if the nucleus lies not eccentrically) on border between halo and plasma of parasite quite sharp outline, which, in my opinion is visible depends on consolidation of the plasma on border of its transition to more liquid halo belonging, perhaps, rather to an a plasma, than a nucleus. Sometimes the nucleus is visible in the form of a stick (apart from the belonging halo); it happens when it is represented as if in a side view; sometimes such stick consists of separate parts. The most part the nucleus settles so eccentrically concerning the parasite that it seems lying absolutely separately, and only the light rim serves as a link between it and plasma of a parasite.

In the sporulation forms (daisy) the nucleus is painted in violet color with more blue shade, than in other stages of a parasite.

Thus pictures are identical with drawings of *Titov, Hentsinsky* and the Italian scientists: only the light point (an endoplazm of *Celli*) of their drawings is stained in violet color. Concerning protoplasm a nucleus is much bigger here, than in separately living parasite. If in a ball 2 parasites, of course, are visible and 2 nuclei. This circumstance is important, in my opinion, in the respect that allows to define quantity of parasites in one ball as at the amyoboid movements the parasite quite often gives such mass of the most various shoots, is frequent, apparently, absolutely not connecting to the main weight that the number of parasites can seem more, than it is actually. Quite often the shoot of a parasite is equal to other weight of it, and only the nucleus allows to solve with positivity, one or several parasites lodged in a ball. Leaving a question of fight of an organism with lodged in it parasites, I can note, that after a fever attack, I found in preparations of blood of adult parasites mostly in a condition of an atrophy, death. I tell it because near young nuclear forms of a parasite adult parasites, the most part pigmented and comprising vacuoles come across; nuclei in them or it isn't visible at all, or instead of the dense, strongly stained nucleus there are separate, untied among themselves, poorly painted, very small grains.

Similar process, as we know, is observed and at an atrophy of cells at the highest animals.

Such parasites with hardly noticeable nucleus I almost didn't meet before an attack of disease.

And so, applying the staining offered by me, it is possible to prove presence of a nucleus at parasites of malaria on dry preparations of blood. That wasn't possible to reach at other ways up to now. I saw a nucleus in a parasite on the same place, as well as the Italian authors.

The difference is only in some particulars that, maybe, depends on that *Grassi* and *Feletti* investigated blood at 4-day fever, and I at 3-day.

As for to quantity of white balls and their mutual relations, it will serve as a subject of other message.

Here I will notice only that hardly at any other disease there are such sharp fluctuations in a quantitative sense, as at malaria fever where at attack height, the quantity of white balls happens less than 3000 on 1 cubic mlm., and before and after an attack reaches 8000 on 1 cubic mlm on the same day.