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BLOOD ELEMENTS IN CERTAIN PISCES WITH SPECIAL REFERENCE TO GALEICHTYS FELIS

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A study of blood cells of *Fundulus majalis, Fundulus heteroclitus,* and *Galeichtys felis* was made with reference to size, shape, and kinds of cells, and with regard to the behavior of the nuclei of the erythrocytes of *Galeichtys felis* as observed in a "Tissue Culture Technique."

This study is a continuation of work done on blood cells of *Rana catesbiana* culture media, at the suggestion of Dr. Ellinor H. Behre. She had observed extrusions of the nuclei in frog blood serum used as culture medium in a study of melanin granules of goldfishes. She found that the extrusions appeared regularly in almost every red cell three to four days after the cultures were prepared. In my study of the frog blood protrusions of the nuclei were observed, but the nuclei never did come entirely out of the cells as Behre had observed. These protrusions also appeared three to four days after cultures were prepared.

This work was done under the much appreciated guidance of Dr. Behre at the Louisiana State University Marine Biological Laboratory at Grand Isle, Louisiana.

In this experiment three fishes were used: *Fundulus majalis*, *Fundulus heteroclitus*, and *Galeichtys felis*. With the first two only studies of fresh preparations, hanging drops, and blood smears were made. Blood cells of *Galeichtys felis* were studied further, using a tissue culture method.

The *Fundulus majalis* and *Fundulus heteroclitus* were collected for the most part with a dip net. These specimens of the *Galeichtys felis* were obtained by hook and line, seine, and trawl in the vicinity of Grand Isle. Only living material was used. The *Galeichtys felis* would live only about five hours after being caught, necessitating prompt utilization upon return to the laboratory. Both species of *Fundulus* were kept alive in the aquarium.

The first step was to make a study of the living blood cells. For this purpose the blood was drawn directly from the heart with a hypodermic needle. Hanging drops were made and temporary mounts were also studied. Some of the latter were stained with Delafields' haematoxylin, eosin, methylene blue, and Chenzinsky's mixture. Blood smears were also made and stained with Ehrlich's and Delafield's haematoxylin.

In making the cultures all of the equipment was carefully sterilized, and all of the glassware scrupulously cleaned with soap, water, alcohol, etc., in the usual manner.

In this work blood cells were cultured in homo-serum. The technique of M. R. and W. H. Lewis was followed as closely as possible.

The following was the procedure. The skin over the heart was wiped off with 70 per cent alcohol and incised so that the needle would not carry in with it any possible contamination from the skin itself. Then with a sterile hyopdermic syringe and sterile needle blood was drawn directly from the heart. It was placed immediately into a cold test tube in cold water; the test tube sealed with parafined lens paper melted in such a fashion that a perfect seal was made. The blood was centrifuged for three minutes with approximately 2000 revolutions per minute. When the serum was ready it was again placed in cold water. Sterile technique was employed throughout and no pipette was used twice. The slides were previously ringed with melted salvaline, using a very fine pipette as a pencil. The slides were labelled with the date and number. The smallest possible amount of serum was dropped from the pipette on the sterile coverslip. Then the smallest possible amount of fresh blood was dropped into the serum. Then the coverslip was inverted, being careful not to touch it with the fingers or to contaminate in any way, and placed over the concavity of the slide so that when pressed down the ring of salvaline was perfect. In this same manner, as many slides as needed were prepared. They were examined microscopically and data recorded.

OBSERVATIONS

A careful study of fresh blood of *Fundulus majalis* showed two principal kinds of cells, white blood cells and red blood cells. Most of the red cells were oval with an oval nucleus, some however were round and contained a round nucleus. The average size was 5.25 microns in length and 3.50 microns in diameter. The average diameter of the nuclei was 4.275 microns. In a hanging drop the white cells were seen to move with ameboid-like movement. The changes in shape were so rapid that before the cell could be drawn as it looked, it had taken on a new appearance. The changes in diameter and shape were so great that measurements were impossible.

These cells stained fairly well with Chenzinsky's mixture. Methylene blue showed the white cells better than any other stain used. The cells stained only slightly with eosin. Delafield's haematoxylin seemed to be the best all-around stain, for it showed both the cytoplasm and nucleus, in both red and white cells.

A study of fresh blood of *Fundulus heteroclitus* showed more variation in shape and size of cells. Some of the red cells were oval with an oval nucleus. The average size of these was 10.50 microns in length and 8.75 in diameter. Some of the cells were spindle-shaped. The largest one of these was 14 microns in length and 5.25 in width. The average was 10 microns in length and 3.50 in width. Some of the cells were round with a round nucleus, sometimes centrally placed, sometimes eccentric. They averaged 10 microns in diameter. Smaller bodies which seemed to be colored with haemoglobin were seen, which we were unable to identify. They might well have been nuclei of cells, the cytoplasm of which was not obvious. The average size of them was 3.50 microns in diameter, which is in accordance with the size of the nuclei. These cells all stained well with Delafield's haematoxylin.

In a study of fresh blood of *Galeichtys felis* white and red cells were seen, the latter showing round, oval and spindle forms. The average of the round cells was approximately 10 microns in diameter. The nuclei of such cells averaged 5 microns in diameter. Oval cells averaged 12 microns in length and 8.75 in diameter. The spindle-shaped cells averaged 10 microns in length and 5 in diameter. The cells did not stain with methylene blue, but did stain with eosin.

Nine successful sets of cultures of *Galeichtys felis* blood cells in homoserum were observed carefully. Cultures prepared on July 9 showed ameboid movement of a white blood cell on the following day observed over a period of thirty-five minutes with constant changes in shape. On the fourth day ameboid movement of another white cell in the same set of cultures was observed. Changes in shape were also noted in a red blood cell during a thirty-five minute period with constant changes, not, however, of an ameboid character. On the third day similar changes in shape of a red cell were observed. These changes were apparently out-pushings of the cytoplasm. Similar changes were observed in another culture in both round and spindle-shaped cells. Beginning July 21 the cultures prepared on July 18 showed the nuclei eccentrically placed. On July 23 the same cultures showed definite protrusions of the nuclei. One such cell was watched from 10:30 a. m. until 5:15 p. m. The nucleus could not be seen to protrude further, but the cytoplasm seemed to become less colored, until it could hardly be seen. On the same day a "red-blood cell" was seen to send out pseudopods. No nucleus was visible. On July 24 a spindle-shaped cell was observed that apparently had sent out a pseudopod, but in seven hours observation no further change in shape was seen. On July 30, observing cultures of July 29, nuclei at the periphery of red blood cells were seen. In one cell the nucleus was almost out of the cell, but was never completely extruded. The nucleus had not moved any on July 31.

Similar results were obtained from the subsequent cultures. Some of the most interesting cultures were sub-cultured, but no further changes took place. Protrusions of the nuclei of the erythrocytes was a fairly constant finding, generally appearing about the third or fourth day, rarely on the second. No definite extrusions were observed even in the sub-cultures. Some of the cultures were kept as long as thirty days.

Attempts were made to culture blood cells of catfish embryos in catfish serum. The embryos available, however, were not young enough to show anything notably different from the mature fish.

DISCUSSION

Maximow found from a study of teleosts that erythrocytes acquire the oval form when they approach the mature condition. Here we found that most of the red blood cells of both species *Fundulus* were oval. The size of the cells observed here agrees with his statement that "the teleosts have relatively small corpuscles, 7 to 13 micra in the long diameter."

The fact that the red blood cells of *Galeichtys felis* were eosinophilic indicates that they were mature, according to the monophyletic school. It would be interesting to see how blood cells of very young catfish embryos would stain. If undifferentiated cells could be obtained and cultured, they might be observed to be progressively basophilic, polychromatophilic, and eosinophilic. Unfortunately material for this study was not available.

The protrusion of the nuclei of the erythrocytes of *Galeichtys felis* in tissue culture is an interesting observation. No definite extrusions were observed is in Behre's finding with *Rana catesbiana*. Our observations are not extensive enough to permit definite conclusions. It is interesting to speculate, however, upon this finding and it does not seem at all unlikely that here in tissue culture we have reproduced the first step in the loss of the nucleus of the erythrocyte comparable to the phylogenetic story.

The next necessary step in such a study is to obtain the cells with protruding nuclei and repeatedly sub-culture to see if any further changes will take place. At least we know from the work done with *Rana catesbiana* and *Galeichtys felis* that something does cause the nuclei to protrude, and that the protrusions are regular.

SUMMARY

1. Average size of the erythrocytes of *Fundulus majalis* was 5.25 micra in length, 3.50 in diameter. Average size of the oval red blood cells of *Fundulus heteroclitus* was 10.50 micra in length, 8.75 in diameter. Average size of the spindle cells was 14 micra in length, 5.25 in diameter. Average size of the oval erythrocytes of *Galeichtys felis* was 12 micra in length, 8.75 in diameter; that of the round cells 10 micra in diameter; that of the spindle shaped cells 10 micra in length, 5 in diameter. These cells of *Galeichtys* felis were eosinophilic.

2. The erythrocytes of *Galeichtys felis* studied in homo-serum were observed to protrude the nuclei regularly three to four days after the cultures were prepared. The protrusions were progressive, beginning with slight displacment of the nuclei to one side. In no case, however, were they found to leave the cell entirely.

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