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Studies on Virus Diseases of Fish. III. Morphological and Experimental Observations on the Lymphocystis Disease of the Pike Perch, *Stizostedion vitreum.*[1]

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(Plate I).

INTRODUCTION AND REVIEW.

The lymphocystis disease of fish is characterized by the appearence of membrane-enclosed uninucleated cells in the connective tissue, especially of the fins. These cells grow to a gigantic size—in *Pleuronectes flesus* for instance, they reach a diameter of 2 millimeters—and enclose in their cytoplasm peculiar reticular bodies which show a staining reaction like basichromatin. They were first supposed to be eggs of parasites (Sandeman, 1892). In a second period they were interpreted as parasitic protozoa and were described by Woodcock in 1904 in the European plaice and flounder as a new species and a new genus of Sporozoa with the name *Lymphocystis johnstonei.* In 1918, J. W. Mavor found a similar disease on the perch *Stizostedion vitreum* in the United States and described the peculiar cells in this fish as a new species: *Lymphocystis vitrea.* But, in the meantime, the writer succeeded in discovering lymphocystis disease on a Baltic Sea race of the European perch *Acerina cernua* and observed the whole development of the peculiar cells (Weissenberg, 1914). A result entirely different from the interpretation of the predecessors was reached. It was discovered that the so-called lymphocystis cells are connective tissue cells of the fish which hypertrophy and become transformed in a peculiar manner by the formation of a membrane and later by the appearance of the conspicuous inclusion bodies.

The study of the morphology and the development of these cells, as well as infection experiments, led to the conclusion that the disease is produced by a virus localized in the hypertrophying cells which are stimulated to a metamorphosis and to gigantic growth.

For the experimental infections, fish of the same Baltic Sea race of *Acerina cernua* were used, on which the disease had been discovered by Weissenberg in a bay of Rügen Island. When fish of this race were kept under laboratory conditions at the Anatomic-Biological Institute of the University of Berlin, Germany, it was seen that healthy perch developed lymphocystis tumors as a rule when kept together with specimens strongly

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affected by lymphocystis disease. Likewise, the disease appeared in a high percentage of such perch after addition of an emulsion of lymphocystis tumor material to the aquarium water (Weissenberg, 1914, p. 801-802; 1921, p. 1365). A similar result was observed with European flounders from the river Warne caught at Rostock, when these were kept in an aquarium infected with an emulsion of lymphocystis tissue from flounders caught at Rügen Island (Weissenberg, 1921, p. 1366).

The decisiveness of these experiments was somewhat impaired by the fact that the treated fish came from waters in which lymphocystis disease is endemic (*Acerina cernua* of Rügen Island) or from waters in which the disease may occasionally occur (flounders of Rostock). Therefore, it was of importance when it was found that in an experiment with fresh water *Acerina cernua* from an inland lake of Germany where the disease was entirely unknown, one of 4 fish developed the tumors after being kept with diseased Baltic Sea *Acerina cernua* (Weissenberg, 1914, p. 802).

Considering the small number of fish in this last-mentioned experiment, it was very desirable to carry out further experiments with a greater number of fish procured from waters in which lymphocystis disease does not occur. This opportunity was obtained with material of the American perch *Stizostedion vitreum* in 1937 when the author came as Visiting Professor of Cytology to Washington University, St. Louis, and Dr. E. V. Cowdry, Head of the Department of Cytology, generously placed the facilities of his laboratory at the author's disposal for a series of experimental observations.

Lymphocystis Disease of *Stizostedion vitreum*.

J. W. Mavor first described in 1918 some cases of lymphocystis disease of the wall-eyed pike perch, *Stizostedion vitreum*, caught at Minocqua, Wisconsin. It is now known that the disease is not rare in this perch caught in Lakes Michigan, Huron and Erie. Roscoe R. Hyde has referred in 1937 in his "Laboratory Outline in Filterable Viruses" (p. 40) to material collected from Lake Erie.

In 1937 the author had the opportunity of seeing fresh material taken from about sixty specimens of *Stizostedion vitreum* caught in Lake Huron and Lake Erie. These were adult fish of a length of 33 to 44 cm. The general aspect of the disease in these fish is very similar to the appearance of the lymphocystis disease of the European perch *Acerina cernua*, described and figured by Weissenberg in 1920. The affected fish show gray transparent tumors, especially on the fins and also often on the trunk and head, forming growths of a diameter of 2 to 3 mm. and sometimes larger cauliflower-like tumors or nodes of the size of a cherry. They are covered by the epithelium and by a connective tissue layer with some pigment cells and contain as the most conspicuous elements the large lymphocystis cells, visible to the naked eye or with the magnifying glass as gray-whitish granules.[2]

Young *Stizostedion vitreum* for Infection Experiments.

On account of the large size of the adult *Stizostedion*, only young fish seemed suitable for experiments in the laboratory. It was very important to procure for experimental infection young fish from a hatchery in a region where lymphocystis disease does not occur. The author is deeply grateful to Mr. E. B. Speaker, State Superintendent of Fisheries, Iowa State Conservation Commission, Des Moines, Iowa, for supplying him with young

[2] The author is greatly indebted for procuring the *Stizostedion* material to Dr. Carl L. Hubbs and Dr. Hilary J. Deason, Ann Arbor, Michigan; to Dr. Thomas H. Langlois, Put-In-Bay, Ohio; and especially to Mr. Robert E. Ellsworth, Supervisor of Spawn Collection, Bay City, Michigan, and to Captain Clarence Smith, Essexville, Michigan.

pike perch from Spirit Lake, Iowa. This lake is fed through a series of glacial lakes located in southern Minnesota and has no connection with the Great Lakes. The young pike perch were raised at the State Fish Hatchery of Spirit Lake and were of pure native Iowa stock.

The young *Stizostedion* of a length of 10-14 cm. were sent in September and October to St. Louis and were kept alive at the Department of Cytology under laboratory conditions for 8-10 weeks. As they were fish of prey, they had to be fed with living food fish (minnows).

Treatment of Young *Stizostedion* from Iowa with Lymphocystis Material of Adult *Stizostedion* from the Great Lakes.

An infection experiment was attempted with 10 young pike perch from Spirit Lake, Iowa. Eleven specimens served as control fish. Lymphocystis tumor tissue removed from adult *Stizostedion vitreum* of the Great Lakes was used as the infecting material. The experimental procedure attempted to imitate the conditions in nature when infection of *Stizostedion* occurs, on the supposition that the lymphocystis virus is picked up either with the food or with the water used in respiration. Tumor material was cut in small bits and dispersed in the tanks. Live minnows were first fed with lymphocystis cells or kept in an emulsion of tumor material and then offered as food to the pike perch. The *Stizostedion* were kept for 13 days under these conditions of exposure to infection. In addition, two of the pike perch with a length of 14 cm. received on the 13th day, by means of a pipette, an injection of a dense emulsion of tumor material through the mouth into the pharynx and through the opercular clefts into the gill atria. The other treated *Stizostedion,* with a length of only 10 to 12 cm., were bathed on the same day in a rather dense emulsion of the tumor material for about half an hour.

Two kinds of tumor material were used, first, material from Lake Erie which was sent without ice and, therefore, arrived in a state of decomposition (material I). Six days later additional tumor material was applied which arrived in excellent fresh condition from Lake Huron (material II). In the previous experiments on the European perch *Acerina cernua* done in Germany, an infection was never recognized before the 9th day of the experiment at the earliest. The close resemblance in morphology of the lymphocystis disease of *Acerina* and *Stizostedion* suggested that the incubation period probably would not be very different for the two fishes belonging to the same family.

Four of the treated fish died within the first 15 days after the experiments were begun, without any signs of lymphocystis infection. But the six surviving fishes developed lymphocystis disease without exception as disclosed by an examination of the fins four weeks later.

Probably only material II, which was applied in much fresher condition than material I, had been effective. Concerning the four fish, which died without showing the eruptions of the disease, two died on the first day of the application of material II, one on the 6th day and the last on the 9th day. Therefore, the period during which these four fishes died, based on the application of material II, would not be appreciably longer than the incubation period observed in the experiments with *Acerina.* Considering the fact that all fishes surviving for a longer period proved to be infected, this may mean that all treated fish developed lymphocystis disease after experimental exposure, when they lived long enough to do so.

Two of the 6 surviving perch which developed lymphocystis cells were the above mentioned larger specimens with a length of 14 cm. These two fish showed much more severe lymphocystis reactions than the 4 smaller *Stizostedion.* A cell count estimating the number of lymphocystis cells appearing

on the tail fin, the favorite place for the localization of the lymphocystis cells in the perch, and for the pectoral fins, showed the following: The number of lymphocystis cells on the tail fin was for the first small specimen 27, for the second 39, for the third 83, and for the fourth 400. On the pectoral fins no lymphocystis cells were observed in the first and second fish, 48 lymphocystis cells were found on the two pectoral fins of the third fish and 35 on the pectoral fins of the fourth fish. In contrast, in each of the larger pike perch, more than 1,000 lymphocystis cells developed on the tail fin. The number of the lymphocystis cells found on the pectoral fins was about 4,000 in the one and an estimated 14,000 in the other larger fish.

The conspicuous difference in the number of lymphocystis cells appearing on the fins of the smaller and the larger fishes may be related to the fact that only the two larger fish were treated with tumor emulsion injected into the pharynx and the gill atria. However, it must not be overlooked that these two, being larger fish, swallowed more food fish than did the smaller, and therefore could pick up with the food fish more of the tumor material.

None of the 11 control fish developed lymphocystis disease. In the six experimentally infected specimens, the growth and differentiation of the lymphocystis cells could be studied during the subsequent period of 33 days. Unfortunately, the pike perch employed in the experiment could not be kept alive longer because of an intercurrent disease which they picked up from the food fish. The last of the 6 experimentally infected *Stizostedion* died on the 66th day of the experiment (the 59th day after treatment with material II).

Summarizing, it can be stated that the experimental infection of *Stizostedion* verifies the previous results of the author on European fishes and confirms his opinion that lymphocystis tumor material is to be considered as highly infectious for fish which belong to the same species as the carrier of the tumors. The conclusiveness of the previous experiments on *Acerina* and *Pleuronectes* was somewhat diminished by the fact that the treated fish could not be procured from waters where lymphocystis disease did not occur and that the number of the fish kept under experimental conditions was very small. The experiments on *Stizostedion*, however, clearly indicate the highly infectious nature of the lympocystis disease by an experimental procedure imitating the conditions which may be effective for infection occurring in nature.

Concerning the Problem of the Control of Lymphocystis Disease.

The lymphocystis disease is not rare in *Stizostedion vitreum* of the Great Lakes. The number of the diseased pike perch caught at Saginaw Bay of Lake Huron was estimated, for instance, in the spring 1937 to be about 5 per cent.

Because severely infected fish have a repulsively diseased appearance and are not saleable, the disease becomes of economic importance.

From a correspondence with Dr. A. S. Hazzard, Director of the Institute for Fisheries Research, Ann Arbor, Michigan, it was gathered that it is a common practice of the commercial fishermen at Saginaw Bay to return to the water any fish, taken in the nets, which are badly affected by lymphocystis disease. The writer had the same experience in Germany with lymphocystis-diseased flounders caught off Rügen Island at the Baltic Sea.

Judging from the present experiments it may be inferred that each lymphocystis-affected perch is highly contagious for other wall-eyed pike perch. It must be strongly emphasized that there is no hope of preventing the spread of this disease except by destroying all affected *Stizostedion* taken in the nets so that no lymphocystis material will return into the lakes.

Review of the Development of the Lymphocystis Cells of *Acerina* and *Pleuronectes*.

Previous observations about the development of the lymphocystis cells of the perch *Acerina cernua* (Weissenberg, 1914, 1920, 1921), gave the following results: On fish kept under infection conditions, groups of connective tissue cells (fibroblasts and osteoblasts) began to hypertrophy during the second week, especially in the fin membranes. Originally these cells were characterized by only a small amount of cytoplasm and by nuclei containing little fluid. Now they became richer in cytoplasm. Their nuclei swelled and their nucleoli increased in size. About one day later numerous hypertrophying connective tissue cells drew in their amoeboid processes, became round in shape and a transparent membrane appeared on their surface. By the appearance of this membrane the cells were characterized as young lymphocystis cells, which now began to grow continuously.

At first they contained no inclusion body. But, about one week later, an inclusion appeared in their cytoplasm as a tiny point which increased in size to a round compact body resembling very much a Guarnieri body of a corneal epithelium cell of the rabbit after inoculation with vaccine virus. The young lymphocystis cells grew larger and larger and corresponding to their growth the inclusion body increased more and more in size to an oval disc, then to a calotte, which became fenestrated and sprouted into a network of basophilic staining reaction. The network lay at first on one side of the nucleus but surrounded the nucleus with its meshes in the following weeks. In the course of one year the lymphocystis cells reached a gigantic size with a diameter of 700 microns and in their cytoplasm the network was distributed with numerous folds.

It is characteristic for *Acerina* that, as a rule, only one network develops from a single inclusion body and that accessory inclusion bodies, if there should be any present at all, remain rudimentary (Weissenberg, 1921, p. 1372). In contrast to *Acerina*, the lymphocystis cells of the European flounder, *Pleuronectes flesus*, always contain as adult cells very numerous inclusion networks. These inclusions appear in young flounder lymphocystis cells as small bodies, one after another (Weissenberg, 1921, pp. 1371, 1373). Another difference between the lymphocystis cells of *Pleuronectes* and *Acerina* is that the nucleus of the *Acerina* lymphocystis cells contains as a rule only one nucleolus, rarer two nucleoli, whereas numerous nucleoli have always developed in the adult *Pleuronectes* lymphocystis cells.

Stages of the Development of the *Stizostedion* Lymphocystis Cells.

Hitherto only adult *Stizostedion* lymphocystis cells have been described (Mavor, 1918). They resemble the lymphocystis cells of *Acerina* in the configuration of the membrane, in the formation of one inclusion network and especially in the structure of the nucleus. This striking resemblance is easy to understand since both fishes belong to the same family, the perches. Judging from the resemblance of the adult lymphocystis cells of *Stizostedion* and *Acerina* it might be supposed that the development of the lymphocystis cells of *Stizostedion* would also take a course similar to that seen on *Acerina*. The stages of development observed in the infection experiment, three of which are pictured on the plate, follow indeed very well the course of development observed on *Acerina*.

Fig. 1 shows the youngest stage of lymphocystis cells observed in a piece of a tail fin border excised on the 29th day of the infection experiment (the 23rd day after the start of the treatment with material II). From a total preparation a group of 11 lymphocystis cells, which developed in the periosteum of a fin-ray, is pictured. The cells can be recognized as lymphocystis cells by their round shape, by the contour of the still thin cell-mem-

brane and by their inclusions (*i*). The cytoplasm of each lymphocystis cell contains one inclusion body which is now at the stage of development in which it resembles very much the Guarnieri bodies of cells of mammals infected by vaccine virus. The inclusions are always surrounded by the halo of a fluid vacuole and when they are projecting in the total preparation over the nucleus, as in cell B for instance, they may be easily distinguished from the nucleoli by this halo. The diameter of the lymphocystis cells varies from 13.5 to 27.4 microns because, as usual, some cells are developing faster while others are lagging a little behind. The two largest cells (F and D) contain the largest inclusion bodies, whereas as a rule small inclusion bodies are seen in small lymphocystis cells, for instance in cell J.

Among the young lymphocystis cells lie naked cells (h) of amoeboid forms which have a denser cytoplasm and usually also a larger size than the normal cells of the periosteum. Apparently they represent hypertrophying fibroblasts which were stimulated to the formation of lymphocystis cells but did not accomplish this development, remaining naked cells without inclusions. The stellate cells to the right from the lymphocystis cells A and D evidently represent some of these originally hypertrophying fibroblasts which were secondarily compressed by the quickly increasing lymphocystis cells.

The pseudopodium-like processes of the lymphocystis cells A and D are to be considered as a deviation from the rule that lymphocystis cells usually appear perfectly round. There is no reason to suppose that these cells could be damaged artificially by pressure. The observations on *Acerina* had led to the opinion that the hypertrophied fibroblasts draw in their amoeboid processes and become round cells before the surrounding membrane appears. But the processes observed on these young lymphocystis cells of *Stizostedion* might be interpreted as pointing out that occasionally the membrane formation of the lymphocystis cells may begin before all the amoeboid processes are taken in and that the irregular shape can remain recognizable for some time.

The lymphocystis cells of Fig. 2 are from a total preparation of a fin border excised on the 44th day of the infection experiment (the 38th day after the start of the treatment with material II). Thus the four cells represent 15 days more of development than the lymphocystis cells of Fig. 1. In these two weeks they have considerably increased in size. The dimension of the smallest cell of this group is 45 by 37.4 microns, the size of the largest cell 67.7 by 58.5 microns. A corresponding enlargement can be seen on the nuclei and the nucleoli. The cell-membrane has become thicker and appears as a glassy, double-contoured layer. A remarkable progress of development is seen in the inclusion bodies which have considerably increased in size. They are seen in the three smaller cells as discs of approximately oval form. In the largest cell the inclusion body (*i*) has become fenestrated and shows a basket-like shape. Whereas the inclusions of the stage of Fig. 1 were rather compact bodies, they display now an alveolar or reticular structure, strongly stained with hematoxylin. The meshes or alveoles are filled with a paler staining substance.

Figs. 3 and 4 represent the latest stage of development which could be observed in the infection experiment. The two lymphocystis cells belong to a total preparation of the tail fin border of a young pike perch which lived about two weeks longer than the other five specimens. The cells were preserved on the 61st day of the infection experiment (the 55th day after the start of the treatment with material II). Thus they represent a stage of development which is 17 days older than the lymphocystis cells in Fig. 2. In comparison to these there is again a considerable increase in size to be seen. The membrane has become still thicker. The cell represented in Fig. 3 with two adjustments (A and B), has with the membrane a size of 81 by 72 microns. The cell of Fig. 4, the cytoplasm of which has withdrawn from

the membrane by shrinking, has at the circumference of the membrane reached a size of 113 by 97 microns.

Along with the enlargement of the cell body the nucleus and the nucleolus have also increased in size. The most conspicuous progress in development is seen on the inclusion body which has grown even faster than the other contituents of the cell. The shape of the inclusion body is now a fenestrated calotte which can also be designated as a network of coarse bars. The bars themselves show many larger and smaller vacuoles which give them an alveolar structure.

As is seen in Fig. 3A, which represents the view of the inclusion network from the side ("en profil") in an optical section through the cell, the network has extended through a considerable part of the cell surrounding the nucleus on one side. Fig. 3B shows a higher adjustment of the same cell.

In Fig. 4 the inclusion network is seen "en face" with medium adjustment. Therefore, only the brim of the calotte of the inclusion network is represented surrounding the nucleus. The processes of the network directed toward the center-part of the picture would appear with high adjustment as continuing into the middle curvature of the fenestrated calotte.

In summarizing it may be stated for the three stages of development represented in Fig. 1, in Fig. 2 and in Figs. 3 and 4 that in the observed period of 33 days the largest diameters of the largest lymphocystis cells have increased from 27.4 to 113 microns. During the same time the inclusion body has developed from the stage of a small, rather compact body, which looks very similar to the so-called Guarnieri bodies of cells of mammals infected by vaccine virus, to a wide network which surrounds one half of the nucleus.

Comparison of Young Lymphocystis Cells of *Stizostedion vitreum* and of *Acerina cernua*.

The general aspect of the young lymphocystis cells of *Stizostedion vitreum*, observed in the infection experiment, and of the corresponding stages of *Acerina* lymphocystis cells is very similar. It is easy to understand why the cell-structure is similar since the two fish belong to the same family, the Percidae. As a rule, the lymphocystis cells of both fish contain only one large nucleolus, occasionally two nucleoli. Often binucleated cells are to be found in *Stizostedion* as well as in *Acerina*.

The type of the inclusions, which usually appear as single bodies, occasionally as double and very seldom as multiple bodies, is also similar in both fish. A slight difference is seen in the stage of the sprouting network, in that the bars of the network appear coarser in *Stizostedion* than in *Acerina*.

More conspicuous is the contrast in the size of the cells of corresponding stages. Whereas the youngest lymphocystis cells of both fish seem to be not essentially different in size, in *Stizostedion* the lymphocystis cells as well as their inclusion bodies grow faster than in *Acerina*. Therefore, the lymphocystis cells in stages which are characterized by large oval inclusion bodies or by the beginning of the formation of the network (Fig. 2), are about one-third larger in *Stizostedion* than in *Acerina*. The same fact can be noted in the following stages in which the extending network has surrounded about one-half of the nucleus (Figs. 3 and 4). Consequently, some morphological details can be demonstrated in *Stizostedion* with the high dry objective, whereas in *Acerina* the oil immersion must be employed.

The statement that the growing lymphocystis cells in corresponding stages are about one-third larger in *Stizostedion* than in *Acerina* harmonizes with the fact that the lymphocystis cells apparently reach a larger final size in *Stizostedion* than in *Acerina*. Whereas the *Acerina* lymphocystis

cells, as a rule, reach no more than 700 microns in diameter, lymphocystis cells of 1,200 by 900 microns could be seen in some tumors of the *Stizostedion* material from Lake Huron. It is still to be determined whether this is the maximum size or if full grown lymphocystis cells of *Stizostedion* can attain still larger dimensions.

SUMMARY.

1. The high infectiousness of lymphocystis-tumor material for fish of the same species was proved in an infection experiment on *Stizostedion vitreum* with the treatment of young fish from a lake where lymphocystis disease is unknown. The experimental procedure attempted to imitate the conditions which may be effective in nature for the infection of *Stizostedion*, on the supposition that the lymphocystis virus is picked up either with the food or with the water used in respiration. The experiment started with 10 treated specimens. Four died early during a period which probably has to be considered as the incubation period. The six surviving perch without exception developed lymphocystis disease.

2. A very severe lymphocystis infection of two of the young pike perch of this experiment may be related to the fact that these fish were of larger size and furthermore were treated with injections of the tumor-emulsion through the mouth into the pharynx and through the opercular clefts into the gill atria.

3. The lymphocystis disease of the American perch *Stizostedion vitreum* shows a great similarity to the lymphocystis disease observed on the European perch *Acerina cernua*. The general aspect of the disease as well as the morphology and the development of the lymphocystis cells which are hypertrophied and metamorphosed fish-connective tissue cells are similar in the two forms. But a difference is seen in the fact that the young lymphocystis cells of *Stizostedion* grow faster than the corresponding stages of *Acerina* and, therefore, are one-third larger. This harmonizes with the fact that the lymphocystis cells of *Stizostedion* can reach 1,200 microns in diameter whereas the final diameter of the lymphocystis cells of *Acerina* is about 700 microns.

4. The growth of the young lymphocystis cells of *Stizostedion* was observed in the infection experiment during a period of 33 days. Within this time the diameter of the largest cells increased from 27.4 to 113 microns and their cytoplasmic inclusions developed from the stage of small, compact bodies, looking very similar to Guarnieri bodies, into wide networks surrounding one-half of the nuclei.

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EXPLANATION OF THE PLATE.

Figs. 1-4. Lymphocystis cells of different stages which developed on the tail fins of young pike perch treated with lymphocystis material of adult pike perch. Total preparations fixed with absol. alcohol 95 parts, acetic acid 5 parts, stained with Delafield's hematoxylin.

General labels: **i**—inclusion bodies, **m**—cell-membrane. × 580.

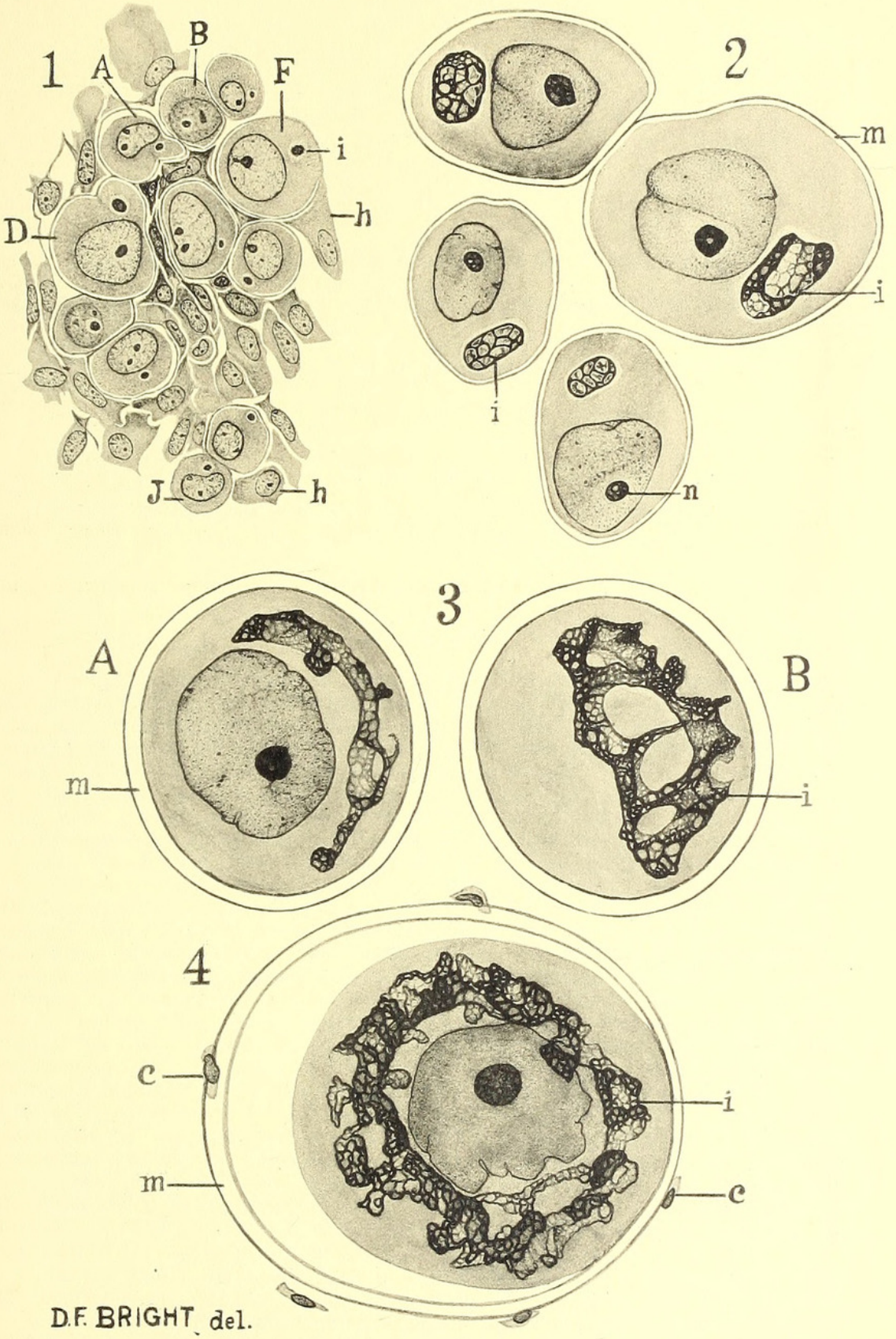
Fig. 1. Stage of the 29th day of the infection experiment. **A, B, D, F, J**—five of the 11 lymphocystis cells of the figured group. **h**—hypertrophied connective tissue cells not transformed into lymphocystis cells.

Fig. 2. Stage of the 44th day of the infection experiment. **n**—nucleolus.

Figs. 3 & 4. Stages of the 61st day of the infection experiment.

Fig. 3. **A**—lymphocystis cell in optical section; **B**—the same cell with higher adjustment.

Fig. 4. Lymphocystis cell with medium adjustment. **c**—small connective tissue cells lying above the membrane.



STUDIES ON VIRUS DISEASES OF FISH. MORPHOLOGICAL AND EXPERIMENTAL OBSERVATIONS ON THE LYMPHOCYSTIS DISEASE OF THE PIKE PERCH, STIZOSTEDION VITREUM.