XVI.—ON THE RETARDATION OF THE DEVELOPMENT OF THE OVA OF THE SHAD (ALOSA SAPIDISSIMA), WITH OBSERVATIONS ON THE EGG FUNGUS AND BACTERIA.*

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Several series of experiments at different times were undertaken by persons connected with the United States Fish Commission having for their object the solution of the following problems: "Is it possible to lower the temperature of the water in which shad-eggs are incubated so as to greatly retard and prolong the process?" "Is it possible to prolong the period of incubation so that large quantities of embryonized ova may be carried for long distances by land or water, so as to effectively stock distant or foreign waters?" These two queries, I think, clearly state the objects of the experiments, and also tacitly indicate the important results which would follow in case practical results should be attained.

That a decrease in temperature would impede or retard the development of ova has been known for a long time, and, without encumbering this essay with references it may be asserted as a truth based on physical reasons and facts. Physiologists and biological philosophers, such as H. Milne-Edwards and Herbert Spencer, have recognized and discussed the influence of fluctuations of temperature on physiological processes. Every genus and perhaps even every species of fishes, in the course of the early development of its ova, appears to present some idiosyncrasy of behavior which demands that its characteristics shall be studied before it is ventured to proceed with experiments of this character. Practically the peculiarities of the ovum of the shad are perhaps as well known as those of any species we are called upon to deal with.

Shad-eggs after impregnation are relatively large, measuring from one-eighth to one-seventh of an inch in diameter. When first extruded from the parent fish they measure about one-fourteenth of an inch in diameter, are somewhat flattened and irregularly rounded in form; the egg-membrane, a true *zona radiata*, is much wrinkled and lies in close contact with the contained vitellus. Immediately after impregnation this membrane becomes tense, is filled with water which has found its way through the membrane from the outside, and is now per-

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fectly spherical, having apparently gained very much in bulk. This gain in size is, however, delusive; it is only the wrinkled egg-membrane which has been distended with water; the vitellus or true germinal and nutritive portion has gained nothing in size. The latter now lies in contact with the lowermost part of the egg-membrane when the whole ovum is at rest, and is always more or less depressed from above in the form of an oblate spheroid. After the germ has been developed, which is discoidal in form and placed on the surface of the vitelline sphere, it usually also occupies a lateral position on the vitellus when the ovum is at rest.

The vitellus rolls about and changes its position inside the egg membrane as the latter's position is altered. The vitellus is heavier than water. A large space filled with fluid now exists between the vitellus and membrane. No adhesive material is found on the outside of the membrane, as in the eggs of the white perch and herring, as may be readily demonstrated with the microscope, although when first extruded they are covered with a somewhat sticky ovarian mucus. The ova are heavier than water and rapidly sink to the bottom of the vessels in which they are undergoing development. All of the hatching apparatus now used for their incubation in water is operated on the principle of a continuous flow, which keeps the ova constantly in motion. So much for the physical behavior and constitution of the shad-egg, which is necessary for the comprehension of what will be said subsequently.

It has been the experience of those intrusted with the work of looking after the artificial incubation of the eggs of the shad that when the temperature of the water was highest the process was completed soonest, and when lowest it took a disproportionately longer time. In illustration of this fact the subjoined data, supplied by Mr. W. F. Page, are of interest, from the records which were kept at the station on the Potomac during the present spring (1881):

	Lot No. 1.	Lot No. 2.	Lot No. 3.
Time in hatching	148 hours.	109 hours.	70 hours.
Average temperature of water	57.2° F.	64.5° F.	74° F.
Average temperature of air	61° F.	66.1° F.	76.25° F.

This series of data shows that with a fall in the temperature of the water down to 57.2° F. it took six days and four hours to complete the development in the egg; with a rise in the temperature of the water to 74° F. the process was complete in a little less than three days. The difference in the times of hatching between lots No. 1 and 3 is 78 hours; the difference in the temperature of the water used is only 16.8° F. Is there a limit to the possibilities of retardation? Experiment has shown that there is. The temperature of ice-water, 38° F., was found to be fatal at the morula or germinal disk stage of development of the shad egg, in the course of experiments made at Havre de

Grace, Md., in 1880. The cells of the germinal disk became brownish, the cleavage furrows obliterated, the disk tended to spread out and become larger across. These phenomena indicated stagnation of development and death. The second series of experiments, conducted by what is known as the "dry method," in a refrigerator box provided with canton flannel trays, devised by Mr. F. N. Clark especially for these experiments, gave better results. We found that the ova merely kept damp on the trays in an air temperature of 52° appeared to develop quite normally, the only serious drawback being the rapid and more or less fatal development of fungus, the mycelium of which would soon grow over the eggs, penetrate the membranes, cause them to collapse, transform the protoplasm of the vitellus into fungus protoplasm, and kill the ova.

The following abstract from my note-book, recording what was observed in watching the results obtained from a trial of Mr. Clark's apparatus, speaks for itself, though it would facilitate the comprehension of the matter if a series of explanatory figures could be introduced:

"Eggs taken June 8 and put into refrigerator at 9 o'clock p. m.; examined June 9 at 9 o'clock a. m.; exposed for 12 hours to a temperature ranging from 54° to 60° F. Cleavage has advanced to the morula stage; *i. e.*, the germinal portion of the egg is still discoidal, lies on one side of the vitellus or yelk, and has not advanced beyond the condition ordinarily reached in three hours with the temperature 72° F.

"Same lot, June 9, 2.30 p. m., advanced but a little beyond the stage just described above; the germinal disk still maintains its characteristics; development normal; temperature 54° F.

"Same lot, June 10, examined at 9.30 a. m.; segmentation cavity developed and blastoderm forming; incipient embryo making its appearance at one side. The blastoderm, however, does not yet cover more than half of the upper hemisphere of the vitellus, a condition ordinarily attained in six hours with the temperature of the water at 72° F. Temperature in refrigerator box now ranging from 52° to 54° F. Eggs of the same age, 36½ hours, in a hatching-jar, have the vitellus completely inclosed by the blastoderm, the embryo formed, with eyes, ears, and brain distinguishable, and the tail is budding out as a small, rounded knob at the posterior end of the embryonic axis, which curves around one side and now extends from one pole of the egg to the other, embracing an arc of 180°.

"Same lot, in refrigerator, examined June 10, at 8.30 p. m., or nearly forty-eight hours after impregnation, shows that the blastoderm has grown down half way over the vitellus, like a hemispherical cap; the keel or carina has been developed. Temperature 53° F. in refrigerator all day. Eggs in a cone of the same age, temperature of the water 65° F., have the embryos well advanced, with the tail free and as long as the portion of the body still in contact with the yelk, but the natatory fold is not developed.

"Eggs which had progressed a considerable way in development, so that the tail was somewhat more advanced than the stage last described, and which did not yet have the eyes pigmented, were also experimented upon at this time. In consequence it was learned that such might be suddenly transferred from the water in which they had previously been undergoing development to the damp cotton-cloth trays without injury from such sudden and continued exposure to an air temperature of 53° F. A most striking fact was that in such as had the choroid or pigmented coat of the eyes in process of development had the formation of the pigment arrested in correspondence with the general arrest of development observed.

"Returning to the eggs of the 8th June, these were examined June 11, 9 a. m. Development is still normal; the eyes are perfecting, but the perfectly normal blastoderm does not yet quite cover the vitellus, the diameter of the opening at the caudal pole, where the vitellus or yelk is still exposed, being equal to about one-seventh of the circumference of the egg. Temperature during the night 49.5° F.

"Other lots of ova, taken on the 6th and 7th of June, and removed from the hatching-cones and put on the cloth trays in the refrigerator box, have been greatly retarded, but the development is normal, no abnormalities whatever having been observed. The lot taken on the 8th and put into the refrigerator on the 9th, after having been in the water for twenty-four hours, are well advanced, the tail being twice as long as the portion of the embryo's body attached to the yelk, and the fin-folds being nearly fully developed dorsally and ventrally.

"The eggs first put into the refrigerator on the evening of the 8th June now show a disposition to wrinkle, *i. e.*, part with the water inclosed between the egg-membrane and the vitellus, and are collapsing. Perhaps this is due to evaporation."

Afterwards I abandoned the view that evaporation was the cause of the collapse and wrinkling of the egg-membranes. I am now fully convinced that it was due to the invasions of a fungus.

"Same lot of eggs of June 8 examined June 11, at 7 p. m. Blastoderm not yet quite but very nearly closed over the vitellus. Only a very small round opening at the tail of the embryo marks the point where its closure is about to take place. Temperature 53° F. in refrigerator. Development normal in those which are not collapsing, after remaining seventy hours on the trays.

"June 12, 11 a. m., eggs of June 8 in refrigerator for the most part still alive. Temperature 52° F. Development has been normal up to this point; the blastoderm has closed over the vitellus, and the tail is just beginning to bud out as a rounded knob, as in twenty four to thirty-six hour embryos hatched in water ranging from 80° to 72° F.

"Eggs of June 7, partially developed, have commenced to collapse in the refrigerator box. This appears to be due to the growth of the fungus on the ova.

"June 13, 10 a. m., examined the eggs put into the refrigerator on the night of the 8th. They are now nearly all dead. Those not affected with fungus mycelium still plump and normal in development; caudal knob but a little more prominent than when examined on the 12th, at 11 a. m. Temperature in box 53° F."

We may sum up the result of these experiments as follows:

After a little more than four and a half days the ova of the shad exposed on cloth trays to a temperature of about 52° F. have not advanced further than they would have done in water at a temperature of 80° F. in 24 hours, or in 30 to 36 hours in water at a temperature of 74° to 68° F.

But after four and a half days our embryos have not yet passed through half of their development, so that it would be safe to say that the period of incubation at this rate could be prolonged for nine days, or a period long enough to readily admit of the transportation of ova so retarded across the Atlantic to England, France, or Germany. The bar to our complete success, however, was the rapid and fatal development of the fungus, which is probably a saprolegnious form identical with the one commonly productive of more or less loss in hatching out ova in water in all the forms of apparatus which I have seen used. If attention were directed to a means of destroying the germs of these organisms I think success might be very confidently anticipated. To effect the complete destruction of the spores in the water used, and to prevent their ever coming into contact with the eggs, upon which they lodge, germinate, and grow, are the preventive measures to be adopted. These measures are, I believe, feasible, but may involve some trouble in their execution. The experiments of Tyndall and Pasteur have taught us that it is possible to sterilize any fluid and render it absolutely free from all forms of organic germs by energetic boiling, taking care afterwards to exclude the germ-laden air by means of stoppers of cotton wool, or by hermetically sealing the vessel. Such a method would, of course, not answer in this case, as in sealing up a vessel containing the eggs in sterilized water they would be smothered. The precautions which are practicable, however, are these: (1) Take care to scald and thoroughly sterilize the pans into which the fish are spawned; (2) take care to wipe the spawning fish clean, and, above all, avoid rubbing off the scales or to allow these to drop into the spawn or milt; (3) use only sterilized water to "bring up" or water-swell the eggs; (4) take care to scald out the refrigerator and cloth trays, so as to sterilize these of any germs; (5) it would also be necessary to boil and sterilize enough water to keep the eggs and cloth trays moist during the process of retardation; (6) the sterilized water should be kept tightly covered in a clean vessel; (7) in managing the refrigerator care should be taken in opening and closing it, and in order to ventilate it the opening in the upper part of the chamber for the admission of air should be provided with a filter of cotton-wool; (8) it would be necessary to scald and sterilize new cotton cloths, since these are almost always laden with germs. These precautions observed, with scrupulous care would insure success, as far as the danger from fungus is concerned, in conducting this mode of retarding development.

The second series of experiments were conducted at Washington, in association with Col. M. McDonald, this gentleman having kindly undertaken to aid in the work of experimentation, by means of various ingenious forms of small and convenient hatching apparatus of his own devising, mostly made of glass. The method pursued consisted partly in treating the eggs for some time on the dry principle on trays, completing the incubation afterwards in the glass apparatus fed with water from a coil of tin pipe kept under ice in a refrigerator. This enabled us to maintain the temperature of the water supply at a pretty constant point, ranging from 60° to 63° F. It was necessary, on account of the distance which the eggs had to be transported, to use trays covered with damp cloths, on which the impregnated, water-swollen ova were carried in transit from the spawning grounds. The experiments were conducted in the basement of the Smithsonian Institution, where some of the trays of eggs were placed in a refrigerator and others put directly into the water at the temperature stated above, using the McDonald apparatus. The results of these experiments were of great interest and of considerable value, as giving us data for certain precautions to be observed in the conduct of future work and experimentation, as may be learned from the account of them which follows.

Colonel McDonald found it necessary to devise some ready means of transporting the ova from the spawning grounds over a score of miles down the Potomac. This necessity for an expedient proved that the transportation of ova by the dry method immediately after they had been water-swollen was possible, and that it would answer for long distances. To illustrate: some were kept on the trays in good condition for seventeen hours, in the ordinary temperature of the air, 70° to 80° F., prevailing at that season of the year (July). When the temperature of the air was up to 90° F. it was found that the ova carried on trays and allowed to remain on them would tend to spoil quickly, as *Bacteria* and *Vibriones* were distinguishable on all the spoiled putrescent ova carefully examined under the microscope. It is therefore evident that in warm weather, in transporting ova by the dry method for long distances, it would be necessary to take certain precautions to prevent the access of the germs of such putrefactive organisms to the eggs. Essentially the same method of procedure recommended to guard against the introduction of the spores of the saprolegnious fungus to the eggs would apply here. Such precautions, however, would only be necessary where it was desired to retard the development for a long time, in case it was desired to transport the ova long distances. I think it would be found practicable to carry eggs on trays on damp cloths for a period of twenty-four to forty-eight hours without the least difficulty, provided a refrigerating apparatus was constructed in which the temperature could be kept at 60° to 65° F.; below this temperature it would not be safe to go for the ordinary purposes of transportation from the spawning grounds remote from the hatching stations. An important matter to attend to in the application of the above plan will be to effectively scald the cloths which are laid in the trays each time before they are again used, or else they will become the nidus of untold myriads of putrefactive germs which will lodge from the air in dust, and the retention and development of which would be favored by whatever of mucus, dead eggs, egg-membranes, and blood might adhere to the cloths from one time to another.

The putrefactive germs always liable to be conveyed in the impalpable dust constantly suspended in the air of houses in this latitude are consequently much more insidious in their approaches than the germs or spores of the saprolegnious fungus, which ordinarily causes a considerable loss of eggs in the hatching-cones. The eggs attacked by the fungus in the water first turn white; the egg-membrane then shows a disposition to wrinkle or become flaccid; the mycelium or growing stage of the fungus is now in active progress. The mycelium is simply a felted meshwork of branching fungus cells, which appropriates the substance of the egg and completely envelops its membrane. In this stage it is comparatively harmless. Afterwards from the felted mycelium threads club-shaped cellular prolongations grow out, which radiate in all directions like the seeds on a dandelion seed-head. In time each one of these club-shaped heads of the fungus, to the number of hundreds on every affected egg, develop a large number of spores or germs on the inside; directly the end bursts open and the minute spores swarm out of the club-shaped spore-case in great numbers. Each of the spores is capable of independent movement by means of long vibrating filaments attached to it at one end. These wander about in the water, lodge on healthy eggs, and grow on and destroy them, so it is important that infested eggs should be removed as soon as they make their appearance in the hatching apparatus. Kühne and Cohn have shown, however, that a temperature of 140° F. is sufficient to kill the germs of *Bacteria* and other putrefactive organisms, and it is very likely that such a temperature or less than the boiling point of water, 212° F., would be quite sufficient to clear off and kill any fungus germs which might adhere to the pans, trays, and cloths used in the transportation of ova.

The preceding account of the development, destructive growth, and maturation of the spores is from personal observations made on eggs infested with fungus in the hatching-cones on the barges at Havre de Grace in 1880, and it is only introduced here to direct attention to some possible means of staying or mitigating its ravages. I do not pretend to know the species by its botanical name. I leave its identification for the cryptogamic botanists; practically a knowledge of its life-history suffices for our purposes.

The following record of the most salient features of my observations, made in association with Colonel McDonald, is on the whole not as encouraging as the experiment made at Havre de Grace, Md., but it is of value on account of the pathological changes or deformities which it was found were induced in embryos when they were subjected to too low a temperature. Only in the very late stages did they appear to be comparatively free from this influence tending to the production of deformities.

A lot of eggs which had the germinal disk biscuit-shaped and normally developed were placed on trays in the refrigerator in the evening, in an air temperature of 45° F. They were found in apparently normal condition after twenty-four hours had elapsed, but had made little or no progress in development. After twenty-four hours more, or after exposure for forty-eight hours to an air temperature of 45° F. on damp cloth trays, the germinal disk was found to be deformed and dead, being helmet-shaped, with one or two constrictions or furrows running round it. The vitellus or yelk still retained its normal appearance, however, the vitelline spheres being clear, with the protoplasmic mesh-work enveloping them in a normal way. Of the same lot, those which were taken out of the air temperature of 45° F. and put into water at 74° F. hatched out normally in a good percentage, without deformities, showing that a sudden transfer to water at a much higher temperature was not attended with difficulties. The prolonged stay of forty-eight hours of the the same lot in the refrigerator at 45° F. showed that complete arrest of development and death would supervene, and that a profound abnornormal change in the form of the germinal disk would result.

Another series of experiments with eggs kept in a temperature of 64° F. showed the same tendency to retard development as was shown by the Havre de Grace experiments. Embryos of the same age in water at 74° F. developed nearly twice as rapidly.

Other experiments showed that eggs which had been retarded in development at a temperature a little below 52° F. for two days exhibited a tendency to develop abnormally. The abnormal phenomena which were noticed principally affected the notochord or embryonic axial cartilaginous rod, which had a tendency to become bent and twisted, while constrictions were also apt to appear, giving it an irregular, beaded, and generally misshapen appearance. Such deformities seemed to affect only the caudal portion of the notochord, the portion toward the head end of the embryo being normal in its appearance. In this way great deformities of the tail arose, so that in a microphotograph of an embryo two-thirds developed the tail, instead of being gracefully bent flatwise to one side, is abruptly bent downwards and then upwards, so as to be approximately V-shaped as seen from the side.

Sometimes the deformation of the tail would only be noticeable at its extremity; at others the deformed portion of the notochord would extend some way forward over the yelk beyond the point where the tail originated, as it budded out from above the point where the blastoderm closed. In no instance was it observed that any deformity or disturbance of the structure of the yelk took place, or that the epiblastic or hypoblastic coverings of the latter were distorted.

The epiblastic coverings of the tail, however, showed a tendency to crumple and become distorted. It was also commonly noticed that the epiblast showed a tendency to proliferate or throw out masses of cells in the form of irregular knob-like clusters. These increased rather than diminished in size as development progressed. No other structure of epiblastic origin took part in the tendency to become misshapen. The eyes, nasal pits, and ear capsules were normal in every respect. The heart pulsated more slowly than in embryos hatched in water of the usual temperature. This was probably due to the benumbing effects of the low temperature.

When deformed embryos were transferred to water of 74° F. they showed no signs of regaining their normal shape, but, on the contrary, the deformity seemed rather to be aggravated as development proceeded. This was the case also when transferred to water ranging from a temperature of 60° to 64° F. Once established, any deformity in development seemed irremediable by any further stages which might be necessary to complete the developmental processes undergone in the egg.

In the light of these researches, taken in their entirety, it would therefore appear that 55° to 53° F. is about the limit to which we can with safety reduce the temperature in which the ova of the shad will undergo their normal development. This temperature would give us, approximately, nine days as the longest period of incubation attainable, time sufficient, added to the four days required for the young to absorb the yelk-sack, or thirteen days in all, to take embryos to be incubated on the route all the way across the Atlantic, or even as far as the Danube or Black Sea. Even this period may be somewhat extended, since it is possible to retard the absorption of the yelk-sack of the young fish by keeping them in water of 60° to 65° F. A temperature of 55° F. would probably not be injurious at this stage. I have kept the young in water at 38° F. for half an hour without apparent injury. They had been hatched only a short time before. The cold would benumb them, and they would lie quietly at the bottom of the vessel until restored to activity as they were warmed up in water of over 70° F., to which they were at once transferred without harm. The muscular masses at the sides of the body were benumbed, as indicated by the quiescent behavior of the embryos. Tissue metamorphosis would be hindered by such a fall in the temperature of the water. We saw that the cold caused the pulsations of the heart to diminish in rapidity. This abatement in the activity of the forces concerned in the transformation of the stored protoplasm of the yelk into the structures of the growing embryo would be very marked in consequence of subjecting young shad to a temperature of 55° F. By this means, reasoning from what we know of the other phases of development when exposed to like temperatures, the absorption of the yelk might be retarded so as not to be completed for six or seven days. This would give us, added to the maximum period of incubation of nine days at 53° F., a total of fifteen days, a period certainly long enough for all practical purposes in the transportation of young fish for stocking purposes.

I would take this opportunity to remark that it must, however, be borne in mind that the growth of an embryo in the egg is different from the growth of the young animal after it has been hatched and begins to feed. The fish embryo has a store of food, which is inclosed in the yelk-sack, which can scarcely be said even to be transformed; it only suffers a change of place, as particle after particle of the yelk substance is removed and built up into the structures of the growing embryo. This transfer is effected through the blood, and also by apposition from below. The young growing animal in feeding must truly transform the protoplasm which it eats; it must digest it; it is carried into the blood as chyle, and so to all parts of the body to repair the waste incident to the exhibition of life. The two processes, upon careful comparison, are wholly unlike. A fall in the temperature diminishes the rate at which this transfer of the yelk substance to the structures of the growing embryo takes place. The frequency of the pulsations of the heart decreases; consequently the yelk substance which is in contact with vascular sinuses below the embryo is not taken into the blood as rapidly. The result of all this is that the absorption of the yelk is impeded and made to minister to the development and growth in size of the young fish for a longer period.

A few other points and I have done with this part of the subject for the present. Most steamships now use fresh water distilled by an apparatus specially constructed for the purpose. This water, provided the most ordinary care was exercised in the storage, would be well fitted to use in the process of retardation. The eggs carried on the trays ought to be occasionally sprinkled with pure sterilized water. The distilled water supplied aboard steamships answers this description fully, and almost everything is accordingly ready to our hands. To reduce the temperature of the water used in the latter stages of development, when it would be necessary to transfer the eggs to water, say on the eighth day, or after they had been for eight days on the damp trays, it would be desirable to avoid contamination of the water from the ice. To avoid this the water should pass through coils of block-tin pipe, placed in tubs, and kept filled with cracked ice; thus we could lower the temperature to at least 60° to 58° F. The same water might be used several times over, because with care it would be so slightly contaminated with organic matter that putrefactive processes could not go on to any hurtful extent. The low temperature would also tend to arrest any tendency to putrescence. How to maintain a uniform temperature in the refrigerators, so as to guard against dangerous fluctuations of temperature, appears to me to be a matter of some difficulty, because sudden meteorological changes, such as we sometimes experience in this latitude, would influence the working of the apparatus. The best regulator would probably be a faithful attendant. The control of the temperature of the water flowing through coils surrounded with ice is, in the light of experience, a comparatively easy matter, as it has been found that in a coil of a given length the fluctuation in the temperature will not vary more than three or four degrees, if a little attention is bestowed in regulating the flow and keeping a good supply of ice packed around the coils.

The prevention of leakage or loss of water from the apparatus would be entirely overcome, both on board cars and steamships, by the adoption of the closed glass hatching-jars, of various forms, devised by Colonel McDonald. They appear to be cheap, and are very economical of room. There can therefore be no objection to the introduction of the apparatus into vessels and railway express cars on the score that it makes objectionable slop and slush on the floors or decks.

The foregoing, it appears to me, is an approximate solution of the problems which we set out to answer. Whether we are right another season's work ought to enable us to decide practically and finally, as we can now take up the subject intelligently. The preliminary experimental work has been completed.

LATER OBSERVATIONS ON THE RETARDATION OF THE DEVELOPMENT OF THE OVA OF THE SHAD.

The following data supplement and confirm in a somewhat remarkable manner the arguments put forth above. The facts there recorded were the results of experiments carried out with the help of apparatus specially designed to artificially lower the temperature of either the air or water in which the eggs were hatched. The value of the present series of observations depends entirely upon the fact that no artificial means were resorted to for the purpose of lowering the temperature, but that the eggs experimented upon, obtained, as they were, as early as the 9th of April, were, in consequence of the then prevailing low temperature of the water, subjected to no extraordinary or artificial condition arising from the use of a complex water or air cooling apparatus. The temperature of the water of the Potomac during the progress of the incubation of the eggs in question was at times as low as 48° F., but as a rule the water then in use in the McDonald hatching-jars, the apparatus utilized in the experiment, fluctuated only between 50° and 56° F., and even then very gradually, as the variation during any one period of twelve hours was rarely more than 1° F. There was a gradual but very slight rise in the temperature of the water from the beginning to the end of the experiment, which covered seventeen days. This gradual rise was covered by 6° or 7° F., as already stated. The average temperature of the water for the whole period was 53¾° F., which, as we see, was only a little above the "danger point," 52° F., if we may so call it, as indicated by my observations made in association with Messrs. McDonald and Clark last year. The results of this experiment have shown us that it is possible to retard the development of shad ova so as to prolong the period of incubation for a period five times that normally occupied in the process in the height of the spawning season, or for almost fifteen days. During my somewhat extended observations on the eggs of this species no such length of time of incubation has been recorded, nor has any one, to the best of my knowledge, recorded the fact that under such conditions of temperature the progress of the evolution of the embryo was perfectly normal, as was the case in the instance now to be described. Several persons have insisted that shad ova developing in too low a temperature would be found to be imperfect, especially the eyes, which, it was said, did not apparently develop at all. The lowest temperature in which I have seen shad-ova develop normally was 49.5° F., as recorded in my report of the experiments during the spring of 1881. Neither in those nor in the embryos which are the subject of this paper was any abnormality observed in the development of the eyes or optic vesicles.

Now for the history of the progress of the experiment and the ova. The latter were taken at one of the Potomac stations organized upon the plan proposed by Colonel McDonald. They were impregnated on the 9th of April at 7 p. m., and brought to the Armory on trays and spread out on damp cloths by Spawntaker Jones. They were placed in one of the McDonald jars on the morning of the 10th of April, but, unfortunately for the fullest fruition of our hopes, during the night, owing to an accidental occurrence or to the meddlesomeness of some irresponsible busy-body, too large a supply of water was turned on, causing the largest proportion of the eggs to be thrown out by way of the escape-pipe of the jar. What were then left, amounting to probably two or three thousand, had to suffice for the material for this account of their development.

On the 11th of April the temperature of the water was 57° F. It had been about the same or a little lower on the 9th and 10th; the water of the Potomac, from which they were obtained at Ferry Landing, was on those dates as low at 48° F. On the 12th the thermometer indicated a temperature in the hatching apparatus ranging from 50° to 51.5° F. On the 13th the temperature ranged from 51° to 52° F. This was the fourth day, and sketches taken from the eggs at this time showed that the blastoderm was just about to close, a condition ordinarily attained in a temperature of 74° F. in somewhat less than twenty-four hours. On the 14th of April the temperature was 52° to 54° F.; on the 15th, 53° Fahr.; on this, the sixth day, the tail began to bud out. On the 16th the temperature was the same as on the previous day, and the tail had by this time, the seventh day, grown to about one-third the length of that of the just-hatched embryo. On the 17th, the temperature was 53.5° F.; on the 18th, 51.5° to 52° F.; on the 19th, 53° to 53.5° F.; development still normal. On the 20th the temperature ranged from 53° to 54°; on the 21st, 55° to 55.5° F., and about this time, or on the twelfth day, the eyes began to show the first signs of pigmentation, becoming a shade darker than hitherto, verging toward brown. On the 22d the temperature of the water was 56°, falling to 55.5° F. On this, the thirteenth day, a few began to hatch; the eyes were now fully pigmented and normal in their development. On the 23d the temperature of the water was 55.5° to 54° F. On the 24th the temperature was from 54° to 54.5° F. During the 23d and 24th days of April the hatching continued, most of the embryos having ruptured their inclosing membranes on the 24th of April, or the fifteenth day of incubation. On the 25th the temperature ranged between 54.5° to 55° F., and on this date, or the sixteenth day, a few of the ova still remained unhatched. On the 26th the temperature was 55° F.; all of the ova were now hatched, and no abnormalities of any sort were noticed. The embryos, however, were for the most part lost, owing, as I think, to the circumstance that the water was allowed to flow too rapidly and violently through the hatching-jar.

The behavior of the hatching-jar was most admirable, but would have been still better had there been a larger quantity of eggs put into the apparatus. The most meritorious feature of the apparatus is the almost entire non-development of the saprolegnious fungus, which causes so great a mortality in some other forms of hatching contrivances in which all of the ova are not in continual movement. The very gradual, gentle, and continual rolling movement of the ova upon each other in the jar apparently prevents the spores of the fungus from adhering. The cleanliness of the apparatus is also to be commended, whereby the use of skim-nets for cleaning is dispensed with, while the material of which it is made—glass—enables one to watch the progress of development very satisfactorily from the outside of the jar with a hand-glass or pocket lens of moderate power.

On the seventeenth day of the experiment the hatched embryos were in the condition of those normally developed at 70° to 75° F., the yelk being ovoidal, clear, and plump. At the rate at which the development progressed it would take five times as long to absorb the bulk of the yelk of an embryo in a temperature of 53.75° F. as at 75° F., or about twenty-five days. This period, added to the prolonged time of incubation at 53.75° F., would cover a space of forty days, or more than twice the time required to carry embryo shad to the farthest confines of Europe. The probability therefore, is that we have exceeded the lowest temperature practically required for this purpose, 55° F. being a much more favorable and less dangerous temperature than that prevailing during the successful experiment of which we have just given a detailed account.

ON THE RATIONALE OF RETARDATION.

Every developing ovum is made up of certain cellular elements, each one of which is provided with a central nuclear body, which appears in the light of recent researches to be the directive dynamic center of all further changes involved in the successive cleavages undergone by the cellular elements constituting that portion of the egg immediately concerned in the formation of the embryo. The assumed disappearance of the nucleus of the egg has been proved not to take place in the act of impregnation, in not only invertebrate ova but also in vertebrate ones as well.*

The hypothetical assumption of a *Cytode* or *Moneron* stage of development in the ova of all forms by Haeckel does not, therefore, appear to be sustained by facts. These and other known facts, such as the recent observation of the metamorphoses of the nuclei of Rhizopods in the act of division (multiplication) also throws doubt on the existence of the *Monera* themselves, as Hensen has suggested.† Nuclear networks inside of cells, as well as intranuclear networks, seem to be of almost universal occurrence, according to the researches of Flemming, Klein, the Hertwigs, Pfitzner, Fol, and others on animals and man, and by Strasburger on plants. Indeed, so strikingly is this true that Strasburger has been tempted to utter the dictum *omnis nucleus e nucleo*, which in English means that all nuclei originate from pre-existing nuclei, just as formerly Schwann expressed himself to the same effect in relation to the genesis of cells. Such intracellular granular networks extending outwards from the nucleus through the protoplasm enveloping it may be seen well developed in the coarse vesicular connective tissue cells of the American oyster, of which I have mounted preparations. Vastly more complex intranuclear reticuli are found in the nucleus of the unripe eggs of the common slipper-limpet, *Crepidula glauca*. I have seen the granular threads in these undergoing the most wonderful active changes of form. Spindle-shaped nuclei, the opposite poles of which were joined by granular threads, have been observed in the eggs of Elasmobranch fishes by Balfour. These were in the act of division, or in the diastole condition spoken of by Flemming. Œllacher has seen granular threads radiating from the nuclei embedded in the cells of th. germinal disk of the trout in its early stages of development. These

* This disappearance is more apparent than real, and while the nucleus may disappear temporarily it soon reappears, showing that nuclear matter still exists in the cell in a concealed or disguised form.

† A. Brass has recently demonstrated the presence of a denser central body in some of the so-called *Monera* by the use of reagents; this central body he regards as answering to the nucleus, while his studies also show these organisms to be far from homogeneous. Huxley, in 1877, had doubts as to the constancy of this distinctive character of the *Monera*; see p. 73 of his Anatomy of the Invertebrates. The Hertwigs have since shown the *Faraminifera* to be nucleated, and Leidy has shown that a nucleus is not always absent in some types, as in *Biomyxa*, for example.

nuclear transformations consequently occur in the cellular elements of fish embryos. These observations are further supported by the fact that both Brooks and myself have observed undoubted evidence of the rythmical nature of segmentation in fish-ova, which ought to be the fact, since it has been shown that the metamorphoses of the nuclei are likewise rythmical in character.

The metamorphoses or changes in the form and structure of the nucleus, are in large part connected with the genesis of new cells, in the successive acts of cleavage or segmentation; their metamorphoses doubtless also play an important part in the functions of rejuvenescence and depuration of cells, or in the general functions, repair and waste, as well as in the excretory and secretory functions of organs. But in retardation we have nothing to do with these latter kind of nuclear metamorphosis; we are only concerned with the alternate elongation and contraction of the nucleus attendant upon the process of segmentation or the fissiparous genesis of new cells, in which the pre-existing nucleus of a cell, about to divide, elongates, becomes severed into two parts, which become, respectively, the nuclei of two new cells. In the process of cleavage it has been shown that during the act of cleavage the nucleus of the cleaving cell elongates, becomes spindle-shaped; that the opposite poles of the spindle become, respectively, the nuclei of the two new cells resulting from the completed process of segmentation. During the active stage the two poles of the spindle are joined by a barrel or spindle-shaped series of granular threads. When the segmentation is about to be consummated these threads, half way between the poles, are found to have developed nodes or swellings; these mark the point through which the segmentation furrow will pass, so as to separate the old cell into two new ones. The segmentation furrow, accordingly, passes at right angles across the long axis of the spindle-shaped nucleus. As soon as the segmentation has been effected the granular threads are withdrawn from the nodal points at the place where the segmentation furrow severed them, and are finally retracted into what were formerly the two poles of the spindle. These poles are now the nuclei of the two new cells, and as soon as the granular threads are withdrawn towards these new polar nuclear centers the latter become globular and pass into the resting stage. Afterwards they both elongate and go through the same process as here described in the course of subsequent cleavage. This alternate elongation of nuclei into a spindle-form and contraction into a spherical form in the process of cleavage has been called by Flemming the *diastole* and *systole* of the nucleus. They accompany the rhythmical phenomena of segmentation and give us a rational and philosophical interpretation of the phenomena of segmentation. It must, I think, be plain to any one that this is essentially a dynamic process, in which the Artisan of organization almost makes His methods of work visible.

It also affords a scientific explanation of the phenomena of retardation. Inasmuch as we have lowered the temperature of the air and water, the media in which the ova of the shad underwent their development, and find that it is retarded in consequence, we must naturally conclude that the rate of segmentation, upon which the rate of development directly depends, has been in some way interfered with or impeded in its progress. Since we also saw that the rhythmical metamorphoses of the nuclei were directly concerned in the process of segmentation—that in them the *vis essentialis*, essential force of segmentation, really resides—it appears to me that we are also really bound to conclude that the fall in the temperature has affected the activity of this *vis essentialis* of the nuclei, which are retarded in their metamorphoses, in consequence of which the rate of segmentation and development is retarded. This fully and clearly accounts for the resulting prolongation of the normal period of development when the temperature of the media in which the ova undergo their evolution is lowered as much as is consistent with their regular, healthful incubation.

If retardation is possible it ought also to be possible to accelerate development. For centuries it has been the practice to accelerate and maintain the growth of plants in hot-houses and forcing-pits during inclement seasons of the year. This is proof enough, as far as the vegetable kingdom is concerned, that acceleration of the processes of growth, which simply means that the acceleration of fissiparous cellular proliferation or segmentation is here possible. Its philosophy is the same in principle as that of retardation; acceleration is the converse or reciprocal principle as opposed to the former. According to a table given by Mr. R. E. Earll, in his paper on the development of the cod, in the United States Fish Commissioner's report for 1878, page 724, we learn that the minimum time of incubation for the ova of this fish is thirteen days, temperature of sea-water 40° F.; the maximum time, according to the same authority, is fifty days, temperature of sea-water 31° F. Our own experience at Wood's Holl last winter taught us that the development of the ova of the cod was capable of being accelerated, for those in a glass cone near a warm stove hatched out in a shorter space of time (sixteen days) than any others. Our power to accelerate the rate of development of the cod may be of use, as we may thereby be enabled to hatch out a large percentage of ova in a very few days. Whether the young would be as vigorous as those incubated in the natural way remains to be learned.

Acceleration, like retardation of development, is accomplished by influencing the rate of the rhythmical metamorphoses of the nuclei of the cells of the embryo. Accelerate the rate of these metamorphoses and segmentation is hastened so as to cause development to proceed more rapidly. The stimulus is heat, a mode of motion, and we are forced to believe from what has preceded that the nuclear metamorphoses are simply the specific modes of motion of the cellular life centers. The molecules of the nuclear spindles, reticuli, &c., are made to move more or less actively in obedience to the fluctuations in the activity of this external stimulus. All this goes without saying, however, that the protoplasm, which in the case of every cell invests the nucleus, may not also share in the process; it is but natural that it should, because free nuclei, independent of any investment of protoplasm, are unknown to histologists.

Inasmuch as the granular particles of nuclear fibers and reticuli exhibit certain modes of motion which appear to be characteristic in the course of segmentation, and since we find that heat, admittedly a mode of motion, accelerates or retards the motion of living nuclear matter in its segmentational metamorphoses, are we not warranted in assuming both of these kinds of motion to be in a degree correlated and interdependent? The significance of the views here set forth in their bearings upon general physiology and pathology would appear to warrant the belief that we may yet be able to solve some of the knottiest problems in biology. Their practical significance in relation to the problems which have presented themselves for solution to the Fish Commission will also be apparent.