cover glass, breaking of the cover glass, and unless the consistency is just right there is difficulty in pressing down the cover glass evenly, frequently necessitating reheating. The advantage of the hot method is the quick cooling allowing immediate use of the section. The cold mounting method obviates all the disadvantages of hot mounting but requires several days for the balsam to dry. This disadvantage has been overcome by the use of a specially prepared liquid Canada balsam.

The preparation is rather simple and involves no unusual materials. Heat the liquid balsam in an evaporating dish slowly (for several hours) until it has a decided orange color and test for brittleness by placing a few drops in cold water. The balsam should be very brittle when cold. Then pour the molten balsam in cardboard boxes and let stand till cold. When it has hardened break away the cardboard and shave the block of balsam with a razor blade or powder it, then dissolve in a small quantity of xylol. Enough balsam should be added to thoroughly saturate the xylol, and the solution should have the consistency of thick honey. Place in the usual Canada balsam bottle, with the ground glass cover, and it is ready for use. If properly prepared, the solution should harden sufficiently in a few hours to render slides usable. By allowing to set overnight the slides can be safely filed in a vertical position, with no fear of the cover glasses moving.

In petrographic practice this method of cold mounting has proven successful and even after a year's time no ill effects have been noticed in the mounts. It is probable that the use of a liquid balsam which will harden overnight will be of as much value to biological microscopic technique as it is in petrographic methods.

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SPECIAL ARTICLES

BACTERIA AS FOOD FOR VERTEBRATES

THE rôle that bacteria directly play in the food supply of the higher forms of animal life, particularly the vertebrates, is not definitely known. That they serve as an important source of food supply to some of the protozoa has been well established experimentally. There is some evidence that bacterial cells can be utilized as food by some of the many-celled invertebrates. The latest evidence is that presented by MacGinitie, who has demonstrated that certain marine worms can utilize a species of Pseudomonas as food.[1] His report does not indicate that the worm can subsist exclusively on a diet of Pseudomonas. Bottom-feeding vertebrates, such as the tadpole, whose mouth parts have a mopping up effect on the bottom, and even such fishes as the sucker, carp, etc., which feed on organic matter on the bottom undoubtedly ingest great numbers of bacteria. The experiments described in this paper were designed to determine whether such bacteria are utilized as food.

In April, 1932, the freshly deposited eggs of *Rana pretiosa* and *Hyla regilla* were gathered. These soon hatched and served as a supply of tadpoles of the two species. They were kept in pans containing water plants of various kinds.

A few preliminary experiments demonstrated that the larval forms of *Hyla regilla* were much more sensitive than those of *Rana pretiosa* to factors other than the food supply and consequently were abandoned as the experimental animal. Both forms died when the bacterial flora became too concentrated, but the larval form of *Rana pretiosa* resisted this condition better. No attempt was made to determine whether death resulted from the reduced oxygen content or from toxic products produced by the bacteria. The animals in one flask died after receiving the bacterial washings from slants several days old.

The procedure adopted after the preliminary experiments was as follows: The tadpoles were placed in 50 cc tap water in 1,000 cc flasks. A 24-hour mixed culture of bacteria taken from the pans containing the stock of tadpoles served as the food supply. The water in the flasks was changed daily or oftener. Some fatalities occurred when too heavy a suspension of bacteria was placed in the flasks. Part of the time a heavy suspension of bacteria was placed in a flask and the tadpoles permitted to feed during the day when they could be watched. At evening they were placed in fresh bacteria-free water and thus were without food during the night. Successful results were obtained by using the bacteria washed from two to three potato extract agar slants as the daily food supply of 4 to 6 tadpoles. The amount of food needed and the concentration of bacteria resisted varied somewhat with the size of the animals. The tadpoles could be seen feeding on the bacteria as these settled on the glass. The turbidity of the suspension cleared as the feeding progressed. In some of the flasks there was sufficient organic matter introduced with the bacteria for bacterial multiplication. When too many bacteria were present, either as the result of being introduced or multiplying, the tadpoles did not thrive.

The first series of flasks were started on April 26, 1932. One flask received only tap water and served

[1] G. E. MacGinitie, "The Rôle of Bacteria as Food for Bottom Animals," SCIENCE, 76: 490, 1932.

as the control. The second flask received the bacteria washed from the surface of agar slants.

The control animals were soon found to be smaller in size and darker in color than the experimental animals. Their fecal matter was less in volume and darker in color. All were dead of starvation in 15 days.

The experimental animals appeared to thrive as well or better than in the stock pans. At the end of the 9th week when in the two-legged stage all died suddenly. The cause of death is unknown, but it was evidently not due to lack of a proper diet. The animals had flourished for nine weeks and died too suddenly.

The second series of animals were taken from the stock pans on June 22. These animals were approximately three months old, about two months older than the first series but from the same batch of eggs. The animals were placed in four flasks. One flask served as the control, receiving nothing but tap water. A second flask received bacteria washed from the surface of two or three standard agar slants. A third flask received similar bacteria washed three times in tap water to remove all organic matter that came from the agar slants. A fourth flask received six to ten drops of potato extract broth and no bacteria.

The tadpoles in the first flask of the second series died of starvation within 20 days. The animals in the second flask developed normally within two months to the four-legged tailless stage and were then preserved in formalin.

The animals in the third flask, feeding on bacteria free of all extraneous organic matter, did not at first thrive as well as those in the second flask. Since some bacteria were lost during the washing process it was thought this might be due to the smaller quantity of food received. The volume of washed bacteria given was increased with the result that the animals began to grow more rapidly and completed a normal metamorphosis into the tailless stage within three months. They were then killed, and preserved in formalin.

The animals in the flask receiving only sterile broth were able to live and grow slowly. Bacterial growth occurred in the flask after the small amount of broth was added. It appears probable that the growth was determined solely by the bacteria developing and not by the other organic matter present. Several of the animals died, but one reached the four-legged stage at the end of the fifth month.

DISCUSSION

The experiments described demonstrate that bacteria serve as a satisfactory food supply for the larval stages of the frog *Rana pretiosa*. Apparently water bacteria contain all the food factors necessary for the metamorphosis of this animal. The animals developed as fast or faster than did the controls in a laboratory environment containing various kinds of water plants. The development of the animals in their natural environment in the streams and ponds was not checked with the growth in the laboratory. A casual examination of the streams seemed to indicate a more rapid development than in the laboratory. Factors other than food were involved.

I am not aware that the larval frog requires vitamins in its food. If it does, then the bacteria used contain them in sufficient quantity for development of the tadpole to the frog stage. The study of vitamin production by bacteria has just begun. It has been shown that vitamins A and B are produced by some but not by all bacteria.[2] In this laboratory we have shown (unpublished data) that *Bacillus acidophilus* does not produce vitamin B in fermented milk. Apparently the occurrence of vitamins in bacteria will be found to be as irregularly distributed as in the higher plants.

In experiments to determine the value of bacteria as food for the higher animals the possibility of one-celled animals interfering by feeding on the bacteria and in turn serving as a source of food must be considered. Apparently protozoa did not multiply in our flasks and affect the results.

CONCLUSIONS

Common water bacteria contain all the food factors, including vitamins, necessary for the normal development of the larval stage of the frog *Rana pretiosa*. Since the larval frog can utilize bacteria as food we should expect to find a wide variety of both invertebrates and vertebrates taking advantage of this source of food supply. Bacteria serve as a direct as well as an indirect factor in determining the food supply of the higher animals.

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LOCALIZATION OF SPIROCHETA PALLIDA IN THE BRAINS OF RABBITS FOLLOWING INTRASPINAL INJECTION OF TESTICULAR TISSUE AND PHYSIOLOGICAL SALT SOLUTION

THE experimental study of neurosyphilis is seriously hampered by the fact that we do not possess a suitable laboratory animal for experimentation. It is an interesting fact that the rabbit, so admirably suited for the study of other manifestations of syphilis, is, generally speaking, incapable of harboring a syphilitic infection of the central nervous system. In spite of persistent efforts of numerous investigators, it has been so far possible to detect the presence of spirochetes in the

[2] C. E. Skinner and M. F. Gunderson, "Production of Vitamin A by a Species of Corynebacterium," *Jour. Biol. Chem.*, xcvii, 53: 1932.