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.

VICTOR BURKE

STATE COLLEGE OF WASHINGTON

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.

central nervous system of syphilitic rabbits only in a few isolated instances. A new approach to the problem was indicated by the discovery of Kolle and Schlossberger[1,2] of the asymptomatic syphilitic infection of mice and rats. These investigators showed that mice and rats infected with pieces of chancre tissue of syphilitic rabbits, were capable of harboring an asymptomatic infection which could be demonstrated by injection of emulsions of their internal organs, including the brain, into normal rabbits, which thereupon developed syphilitic lesions. It was further shown by Schlossberger[3] that spirochetes which have thus passed through the brain of rats or mice acquired the unusual property of being able to penetrate into the brain of rabbits, a property which is maintained through successive passages from rabbit to rabbit. In these experiments and similar experiments of the authors,[4,5] it became possible for the first time to cause the *Spirocheta pallida* to penetrate into the brain of rabbits in a large number of cases.

The affinity of the *Spirocheta pallida* for testicular tissue, as shown, for example, by the experiments of the authors[6,7] in which spirochetes, introduced intraspinally into rabbits, quickly disappeared from the cerebrospinal system and localized in the testicles, suggested an interesting possibility in connection with the so-called Reynals factor. It was shown by Duran-Reynals[8,9] and Hoffman and Duran-Reynals[10] that extracts of certain organs (testicles, kidney, brain and skin) have an enhancing action on the development of vaccine virus and staphylococcus infection and that testicular extract is by far the most active. It was further shown that testicular extract of the rabbit caused injected India ink and Prussian blue to spread much more extensively through the intercellular spaces than was the case when suspensions of Ringer's solution only were used. It is possible that the enhancing power of the testicular extract is accompanied by an effect which consists in rendering the cells more easily penetrable by the injected agents.

It now appeared promising to the authors to attempt to render the central nervous system of the rabbit more easily penetrable to spirochetes by injection of normal testicular extract. This was, in fact, done in a series of experiments of which the present article is a preliminary report. Six rabbits received an intraspinal injection of an emulsion of testicular tissue of normal rabbits. Shortly thereafter they were inoculated, 3 intratesticularly and 3 intravenously, by the usual method with syphilitic testicular tissue (Nichols strain). Six to seven weeks later testicular lesions appeared, and when the brains of the animals were removed and emulsions prepared from them were injected into testicles of normal rabbits, positive results (lesions containing numerous active spirochetes) were obtained in all the six animals. The situation, however, was complicated by the somewhat unexpected fact that a positive result was also obtained with one animal injected intraspinally with physiological salt solution. This renders the interpretation of the results obtained somewhat uncertain. In order to determine whether the effect may not possibly be due to the traumatic shock of the injection, experiments are now in progress in which distilled water and also a number of other substances are being injected intraspinally.

The net result, so far, is that spirochetes may be induced to penetrate into the central nervous system of rabbits with the aid of an intraspinal injection of normal testicular tissue, or (in one case) physiological salt solution.

In conclusion, we take pleasure in acknowledging a very stimulating discussion with Dr. Duran-Reynals which gave the immediate impetus to the experiments here reported.

GEORGE W. RAIZISS
UNIVERSITY OF PENNSYLVANIA
M. SEVERAC
DERMATOLOGICAL RESEARCH LABORATORIES
PHILADELPHIA

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