mere isolation of this organism from the suspected feed or intestinal tract or spleen of a dead animal is insufficient to support the diagnosis of botulism." There is always the likelihood that tissues may be invaded after death by this saprophytic anaerobe which, in the form of a few adventitious spores, may have been making harmless passage through the alimentary tract at the time of the creature's death, brought about by factors that may have been wholly foreign to botulism.

Demonstration of toxin in the field, therefore, in concentrations lethal to birds and in, or closely associated with, likely food items, constituted the primary objective of the past season's study of duck sickness conducted by the Biological Survey in southern Oregon and northern California. It was the logical sequel to the progress made in 1929 and 1930, and it constituted the final unforged link in the complete chain of evidence. Without such toxin demonstration the concept of botulism being the cause of duck sickness still would remain, at least to the bacteriological world, an unproved theory.

It is gratifying to be able to announce that this primary objective was attained, not once, but in at least twenty different instances during the summer of 1931. In each case toxin was demonstrated by the feeding or inoculation of experimental birds (mainly pigeons) with material obtained directly from field sources. Necessary toxin-antitoxin tests were made on every occasion, definitely identifying the toxin as that originating from *C. botulinum,* Type C, of Bengtson.

The media in which toxin was demonstrated included the bodies of birds dying of duck sickness, mud in the immediate vicinity of such bodies, water from shallow pools in infected areas, living and dead larvae of sarcophagid flies, submerged barley and other grains that had lain on mud flats where they were subjected to high temperatures and an alkaline environment. Under conditions that were strictly experimental but which might easily occur in the field, toxin also was demonstrated in a mixed mass of insect remains, copepods, snails, algae and Lemna. The organism, but not its toxin, was also demonstrated in such apparently suitable toxin-producing media as the dead larvae and pupae of hydrophilid beetles, submerged wheat heads of the season's crop, colonies of dead copepods, and miscellaneous insect débris, drifted ashore by wave and wind action.

This array of demonstrated toxin-producing media, though admittedly only a beginning, already includes food items that would indicate the channels through which ducks and shore birds may contract the trouble. Added research we feel is destined to extend the categories of toxin-producing food items of these two groups of highly susceptible birds and, bit by bit, round out our knowledge of the sources and vehicles for the toxin now known to affect more than sixty species of wild birds.

During 1931, progress also was made in the study of other aspects of this problem. Mentioning only a few, attention may be called to the apparent and doubtless important correlation between the incidence of duck sickness and alkalinity; the presence of Type C to the almost if not total exclusion of other types of botulism in duck sickness environments; the degree of susceptibility of lower organisms, both vertebrate and invertebrate, the death of which in the course of an outbreak of the disease would greatly augment the quantity of toxin-producing media; the toxin-destroying properties of high concentrations of certain alkaline salts and the possibility of thus explaining the absence of duck sickness from certain areas otherwise suited to its occurrence. These and numerous other aspects of the problem, mainly bacteriological in character, bid fair to make future studies of duck sickness of as great importance to the bacteriologist as to the conservationist of wild life.

During the season of 1931 Mr. M. F. Gunderson, employed by the Disease Investigation Project of the Biological Survey, worked with the writer, and to him is due great credit for the progress made along bacteriological and toxicological lines. At present he is continuing these studies in the department of bacteriology and immunology, University of Minnesota. It is a fruitful field for pure research as well as for the attainment of results of great practical value in the preservation of wild birds, and it stands to reason that, before the story of western duck sickness is completed, earlier concepts of the disease as well as the prevalence, range and economic importance of *Clostridium botulinum,* Type C, will be greatly changed.

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BIOLOGICAL SURVEY,
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