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WESTERN DUCK SICKNESS, A FORM OF BOTULISM.

KALMBACH, E. R. ; GUNDERSON, M. F.

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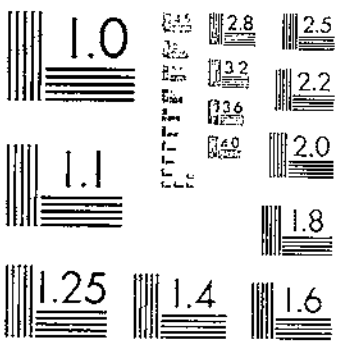

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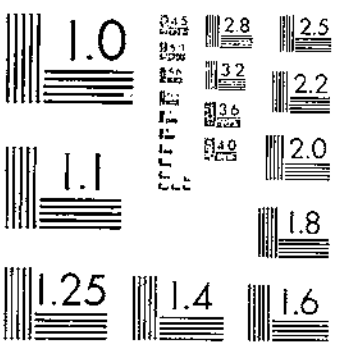

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WESTERN DUCK SICKNESS
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By

E. R. KALMBACH
Senior Biologist, Division of Food Habits Research
Bureau of Biological Survey

WITH BACTERIOLOGICAL CONTRIBUTIONS

By

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UNITED STATES DEPARTMENT OF AGRICULTURE, WASHINGTON, D.C.

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WESTERN DUCK SICKNESS: A FORM OF BOTULISM

By E. R. KALMBACH, *senior biologist, Division of Food Habits Research, Bureau of Biological Survey;* with bacteriological contributions by MILLARD F. GUNDERSON, *Department of Bacteriology and Immunology, University of Minnesota.*[1]

CONTENTS

INTRODUCTION

Duck sickness, a malady affecting not only ducks but also many other groups of wild birds, is today recognized as an outstanding menace to migratory waterfowl in the Western States. The mortality among ducks, coots, gulls, and shore birds of many kinds has been so severe at some points during some years of the past two decades that other factors tending to reduce the numbers of migratory birds in these areas have appeared insignificant (pl. 1, *A* and *B*). In 1910 the extensive mortality among birds in the marshes bordering Great Salt Lake, Utah, first attracted nation-wide attention, and during the years immediately following, the death rate, though reduced, still indicated heavy inroads on western waterfowl. In 1914

[1] A full report of Gunderson's researches, Studies on Western Duck Sickness, a Disease of Wild Water Birds Frequenting Alkaline Lakes, based on work done at the Bureau of Biological Survey laboratory at Klamath Falls, Oreg., during the summer of 1931, and subsequently at the University of Minnesota, was submitted to the graduate faculty of that university as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy, in June 1932.

the Bureau of Biological Survey inaugurated its first study of the malady, and continued the work in 1915 and 1916. A preliminary report (*70*)[2] in 1915 by Alexander Wetmore, followed by his more extensive publication (*71*) in 1918, summarized the findings to that time. The conclusion reached in that investigation, carried out primarily in the highly saline environment of Great Salt Lake and its contiguous alkali flats, was that "duck sickness in Utah is caused by the toxic action of certain soluble salts found in alkali."

Subsequent years brought to light evidence of the disease at other widely scattered western points, in an area extending from the southern Canadian Provinces to Mexico, and from southwestern Minnesota to the west coast (fig. 1, p. 32). In the main, the distribution of the malady conformed to the region of alkaline waters and soils, yet there appeared no close relationship between the prevalence of the disease and either the concentration or the character of the alkali. There were reported from time to time severe outbreaks in areas in which the supposedly causative salts were present in what appeared to be negligible quantities; conversely, certain bodies of water that held high concentrations of the suspected salts served as havens for waterfowl over considerable periods. Though, in general, outbreaks were associated with periods of high temperature and low stages of water (which logically might be interpreted as indices to a condition of greater alkalinity or salinity), the suddenness with which some outbreaks began and ceased found no corollary in the saline conditions current at the time. Differences were noted also between the general syndrome of duck sickness and its pathology, and that of the toxic action of the suspected salts, as further information became available in the growing literature of that field of toxicology. As time went on, other evidence, much of it less convincing in character, increased a feeling among many sportsmen and other observers of wild life that the cause of duck sickness was not yet definitely established. There were some misgivings also among scientific groups, in view of the accumulating evidence, which indicated, at least in areas distant from the highly saline environment of Great Salt Lake, that some factor or factors other than alkali might be involved, or that several maladies having closely similar symptoms might be responsible for the losses.

To clarify these uncertainties, the Biological Survey renewed its study of the disease in 1927. The studies were not continued in 1928, as there was no marked outbreak until late in fall, but in the following year, 1929, investigations again were resumed and were continued through the summer seasons of 1930 and 1931.

The results, which have removed the problem of duck sickness from the field of chemical toxicology to the realm of bacteriology, are presented in this bulletin. The disease has been associated definitely with the toxin of a common saprophitic bacterium, *Clostridium botulinum*, type C, an organism best known in the United States as a frequent cause of limberneck in poultry and forage poisoning in livestock.

As in other kinds of research, much of the success attained in this study has been dependent upon the facilities for doing the work.

[2] Italic numbers in parentheses refer to Literature Cited, p. 78.

Although field experimentation was conducted far from the fully equipped laboratory that has come to be looked upon as a matter of course in bacteriological research, deficiencies in this respect were compensated for by help of inestimable value rendered by many individuals and organizations interested in the success of the investigation.[3]

SCOPE OF THE PRESENT INVESTIGATION

The first intensive study of the present investigation was that conducted in 1927 by C. C. Sperry, of the Bureau of Biological Survey. With headquarters at Klamath Falls, Oreg., he made observations in the adjacent area throughout August and September. Although no laboratory facilities were available at that time, Sperry constructed a number of cages in which he conducted his experiments. One of these, of particular significance, is mentioned on page 56.

In the following year (1928) studies were not resumed, since duck sickness did not appear until too late in the season to warrant dispatching an investigator and providing equipment for a period of study that would necessarily be brief. In 1929 actual investigative work was begun at Klamath Falls on June 8 and continued until October 21. In that season a field laboratory (pl. 6, *A*) was established and additional experimental pens were constructed. The laboratory afforded facilities for microscopic work and the preparation of histological specimens, limited chemical analysis of waters, and experimentation in other ways. Attempts were made to produce the disease by feeding birds with natural and synthetic "alkalis"; feeding experiments were undertaken with body tissues of birds dying of the disease, with fermented vegetable matter, and with sarcophagid-fly larvae; and, even in that season, experiments were conducted to determine whether botulism might be the cause of the disease.

Through a cooperative agreement with the Bureau of Animal Industry, Eloise B. Cram, of the Zoological Division, was detailed to the Klamath Falls laboratory from August 5 to September 3, 1929, to study the relation of internal parasites to duck sickness. A résumé of Dr. Cram's findings is presented on page 10. The late C. Dwight Marsh, of the Pathological Division of that Bureau, also inspected the Klamath Falls district (Aug. 25–27, 1929) to learn whether toxic algae might be involved. In the same season

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[3] J. C. Boyle and H. P. Bosworth, Jr., of the California-Oregon Power Co., at Klamath Falls, Oreg., kindly made available a 1-room concrete building for use as a laboratory, and land on which to construct experiment pens, and gratuitously supplied electric light and water. To Supt. B. E. Hayden and other representatives of the Bureau of Reclamation at Klamath Falls thanks are due for data on local temperatures and water levels, factors playing an important part in the incidence of duck sickness. Helpful advice in the pursuit of the bacteriological and toxicological aspects of the research was given by Robert G. Green, of the Department of Bacteriology and Immunology of the University of Minnesota; Ivan C. Hall, of the Department of Bacteriology and Public Health of the University of Colorado; and by L. T. Giltner, of the Pathological Division of the Bureau of Animal Industry. James F. Couch, also of the Pathological Division, and Eloise B. Cram, of the Zoological Division, both of the Bureau of Animal Industry, contributed much to the progress of the work while under temporary assignment at the Klamath Falls laboratory. To W. R. Taylor, of the University of Pennsylvania, thanks are due for the identification of algae. To W. H. Meal, manager of the Bear River Club, Brigham, Utah, acknowledgment is made of historical data concerning duck sickness at Great Salt Lake covering the period subsequent to Dr. Wetmore's work. Gratitude is expressed collectively to members of the Bureau of Biological Survey for aid in many lines of the investigation and to numerous correspondents and collaborators of the Bureau in Canada and Mexico and throughout the United States, who have contributed much through written word and by specimens to the total knowledge of duck sickness in its field aspects.

helpful suggestions were offered by B. T. Simms of the Oregon State Agricultural College.

During the winter of 1929–30 an arrangement was made with the Water and Beverage Section of the then Food, Drug, and Insecticide Administration of this Department for detailed analyses of 28 samples of water collected in the Klamath Falls district. These, together with the analyses of 16 samples collected by Sperry in 1927 and others collected earlier by Wetmore, at Great Salt Lake, form the basis of much of the discussion in this report of "alkali" in its relation to duck sickness. In the spring of 1930 a few experiments were conducted at Washington, D.C., to note the effect of certain lead salts and hydrogen sulphide on ducks.

At the beginning of the field work in 1930 (July 1) little more was known of the real cause of duck sickness than at the inauguration of the study in 1927. Some new evidence of a positive character with respect to the toxicity of "alkali" was brought forward in 1929 by Shaw (*60*), yet there was also an ever-increasing doubt as to the adequacy of this postulate in the light of certain field aspects of the malady. Accordingly, with the object of learning more of the role of alkali as a causative factor in the Klamath Falls district, a cooperative arrangement was effected whereby James F. Couch, of the Pathological Division of the Bureau of Animal Industry, was detailed to the Klamath Falls laboratory for the period August 18 to September 8, 1930. Couch's contribution, discussed elsewhere (pp. 16–17), deals largely with the analyses of waters and muds of the sickness and nonsickness areas.

Late in July and during the early part of August 1930 a series of unexpected results attained in experimentally feeding gulls on the body tissues of birds dying of duck sickness focused attention on this aspect of the study. It became evident that something closely akin to duck sickness was being produced through the feeding of such material after it had undergone a certain degree of decomposition. This naturally revived interest in the concept of botulism, which had been considered in earlier work, but for which no demonstrable evidence had been revealed. Other corroborative experiments followed rapidly, and by early October the presence of *Clostridium botulinum*, type C, had been demonstrated by L. T. Giltner in culture material sent to the Bureau of Animal Industry. In the laboratory at Klamath Falls experimental work during the latter part of that season was directed mainly toward determining the relative susceptibility of different species, the suitability of various body tissues as incubating media, the effect of physical and chemical agencies on the toxicity of cultures, and learning what factors appeared to favor toxin production in the field. Late in 1930 there were published two brief articles, one by Kalmbach (*41*), and the other by Giltner and Couch (*25*), in which the season's progress in the duck sickness studies was set forth.

During the winter 1930–31 additional experiments were carried out at Washington, D.C., using chiefly impure cultures brought from duck sickness areas of the West. This work dealt largely with the matter of toxin production in various media, including grains. Further research was directed toward determining the effect of various salts on toxin and toxin production. Despite the convincing charac-

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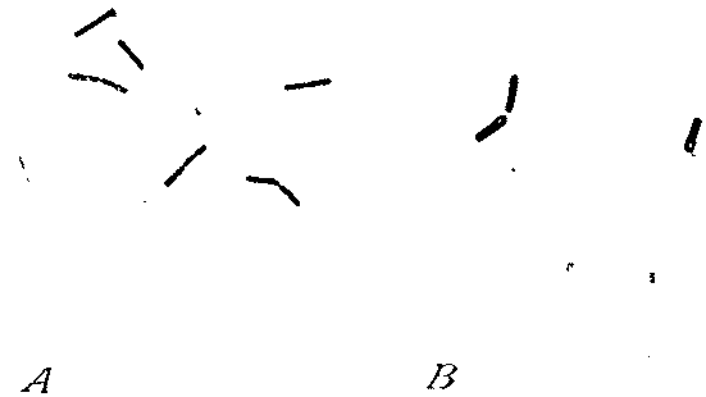
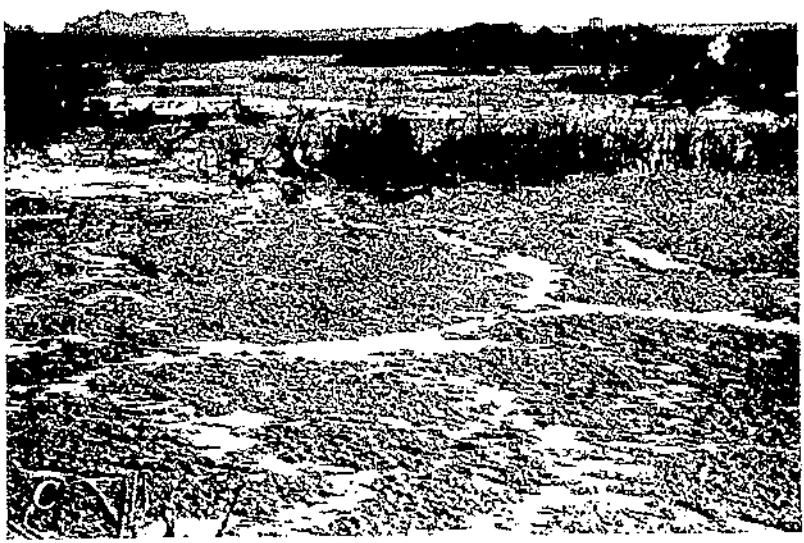
A, Typical duck-sickness environment; shallow, stagnant water and alkaline mud flats. *B*, Severe outbreak of the disease on the south side of Willard Spur, Utah, in 1932. On November 12, for 6 to 8 miles, there were 8,000 to 10,000 dead ducks to the mile of shore line. Many additional thousands lay scattered along the shores of Bear River Bay.





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A and *B*, The causative organism of duck sickness: 1, A group of vegetative forms of *Clostridium botulinum*, type C. *B*, Two showing subterminal spore. Both × about 1,000. *C*, Beds of filamentous algae play a part in the incidence of duck sickness by preventing water movement, thus intensifying local conditions of decay.

ter of the results attained in the experimental work of 1930 there still remained, at the start of field operations in 1931, the necessity of demonstrating that toxin could be produced under field conditions and might be associated with food and water likely to be ingested by birds.

To attain this objective, accomplished during the summer of 1931, M. F. Gunderson was employed under the disease investigations project of the Bureau of Biological Survey. Later Gunderson continued his studies at the University of Minnesota, and the results of his labors have been incorporated in those sections of this report dealing with the bacteriology of the problem. At the conclusion of field studies in the Klamath Falls district (Oct. 8, 1931), Kalmbach visited several duck-sickness areas in California and Texas, where material was collected for subsequent culturing and identification.

THE CAUSATIVE ORGANISM, CLOSTRIDIUM BOTULINUM, TYPE C

Duck sickness (botulism) is caused by the toxin, or poison, elaborated by a micro-organism technically known as *Clostridium botulinum*, type C, and is, in fact, a form of food poisoning. This micro-organism is a relatively large, rodlike bacterium, measuring 3μ to 8μ in length and 0.5μ to 0.8μ in width, and frequently may be found growing in short chains of two or more bacilli connected end to end (pl. 2 *A*). After a period of growth in a suitable medium many of the bacilli form spores, indicated by a swelling of the rod near one end (pl. 2 *B*). It is as a spore that the organism may persist from season to season or even for years, to develop, multiply, and evolve its toxin again when conditions are suitable for growth.

Four principal forms, or strains, of this organism are recognized, designated as types A, B, C, and D (*48*). Types A and B, of wide distribution both in the New and Old Worlds (*46*, *47*) are the ones involved in human botulism. Their toxins are among the most deadly known. Both have entered the picture of human medicine through the generation of their toxins in imperfectly preserved foods, and when these foods have been made available to domestic birds and mammals, these too have succumbed. Chickens, however, are relatively resistant to the toxin of type B.

Type C, the form involved in the present study, also is of wide distribution and, of all the forms, plays the most important role in the destruction of wild and domestic birds and animals. In North America it is the cause of duck sickness in wild fowl, forage poisoning among livestock, and a certain mortality among domestic poultry (*3*, *26*); in Australia and Tasmania this, or a very closely related form, is the cause of the malady known as bulbar paralysis, or midland cattle disease (*58*); in South Africa, where it is known to affect cattle, horses, goats, domestic ducks, and even ostriches, it is the causative factor of the dread lamsiekte (*65*, *66*). It is reported to occur in Europe (*47*), and if the malady among wild fowl reported by Wetmore in Uruguay (p. 32) is due to the same organism, South America also must be included in its broad range. Two variants of type C have been designated as C_α and C_β, by Gunnison and Meyer (*31*) on the basis of certain bacteriological differences.

The form responsible for duck sickness in this country recently has been determined by Gunnison and Coleman (*29*) to be of the variant C_α.

Type D, closely related to type C, occurs with the latter in South Africa, also as a causative factor of lamsiekte among cattle.

Whereas the originally described form of *Clostridium botulinum*, apparently a type B form, has been known since 1896 (*20*) and the literature dealing with it and the other human strain, type A, is voluminous, our knowledge of type C, now demonstrated to be the cause of duck sickness, dates only from 1922, when Ida A. Bengtson, of the United States Hygienic Laboratory, isolated and described it from the larvae of carrion flies (*2*). In 1924 she published a more extensive paper dealing with this particular strain (*3*), but at that time no evidence had come to light indicating that the organism was connected in any way with duck sickness.

Although the four types mentioned differ one from another in the manner in which they grow in various culture media; in the effect of their toxin on different animals, including man; in their reaction to the antitoxins of the respective types; and in other ways, all require essentially the same conditions for growth and development of their toxin. All are known as anaerobes, that is, organisms that thrive only in media or under conditions from which oxygen has been excluded. Likewise all are saprophytes, living and producing toxin in the presence of dead organic matter, animal or vegetable. In other words, the various forms of *Clostridium botulinum*, including the type that produces the duck sickness, are organisms associated with decay, and their toxins are in a sense products of decay. It must not be inferred from this, however, that all decaying material is toxic—only that infected with *C. botulinum* (and a few other organisms not pertinent to this discussion) need be viewed with concern.

In distinction from the causative factors of certain infectious diseases, bacteria of the species *Clostridium botulinum* do not thrive in the living body, and their rodlike bacilli or the minute spores, washed free of all toxin, may even be eaten by birds (at least in doses that are not excessive) without ill effects. It is the preformed toxin, developed outside the body of the bird, and that alone, that produces the disease. The malady can be contracted only through food or water assimilation. It cannot be imparted from one living bird to another. For this reason duck sickness is not contagious.

The extent of toxin production is controlled by a number of factors and conditions. There must, in the first place, be a suitable medium and a deficiency of air (oxygen). Temperature must be kept within certain limits: Earlier investigations with types A and B *botulinum* revealed a temperature range from 28° to 37.5° C. (82.4° to 99.5° F.) for rapid toxin production (*5*), but later studies placed the optimum for type C, derived from duck-sickness cases, at 37° C. (*29*). An alkaline condition is looked upon as being more favorable to growth than one that is neutral or acid. At the same time preformed toxin may be affected adversely in nature by such factors as extreme alkalinity or salinity, light, dessication, or air. In the laboratory a temperature of 80° C. (176° F.) maintained for 30

minutes destroyed the toxin of a type C strain, and boiling for an hour destroyed most spores (*3*).

Dispersal of the organism may take place in many ways. Sarcophagous insects are common vectors; moving water easily may transport organic matter laden with the bacteria or their spores; and even the winds may carry particles of soil or other material to which the microscopic spores may be attached. Birds themselves, because of their frequenting infected areas, may transport the organism with mud or vegetation adhering to feet or plumage. It is even likely that ducks that have ingested lethal doses of the toxin may continue on their migration for many miles before incipient paralysis halts their progress. Then, should they die, their decaying bodies, acting as media, may become centers of other areas of infection. Various wild and domestic mammals, as well as man himself, also may become accidental carriers of the organism in traveling from place to place.

EARLIER THEORIES AS TO CAUSE

The expression "earlier theories" is applied to all concepts of duck sickness prior to the demonstration of botulism by the present investigation. With respect to some of these concepts, particularly those involving infectious bacterial disease, parasitism, and "alkali" toxicity, the Bureau of Biological Survey, in cooperation with other bureaus of the Department of Agriculture, has made considerable contributions, both in its earlier studies (1914–16) and in those of the present investigation previous to August 1930.

INDUSTRIAL WASTE AND POLLUTION

Among the earliest of the theories advanced to explain duck sickness was that of chemical pollution of waters by industrial waste. It was surmised that sulphur fumes from the great smelters near Salt Lake City were absorbed by the waters of streams and marshes to an extent sufficient to make these toxic to birds. The theory was weakened by the fact that duck sickness has occurred in typical and virulent form in areas where smelters do not exist. Furthermore, the syndromes of intoxication by sulphurous and sulphuric acids and by arsenic (another element credited with the cause of the disease), and the pathological lesions produced by them, do not correspond with those of duck sickness. Conclusions in this respect are based on experimental work in 1915 by Wetmore (*70, p. 5*) at Great Salt Lake as well as on the researches of toxicologists elsewhere. Attention may be called to the prevalence of the disease in such environments as Lake Newell, Alberta, Lake Malheur, Oreg., Tule Lake, Calif., and the "tanks" in the Panhandle of Texas, as an indication that, at least in those localities, industrial pollution plays no major part in the production of the disease.

Similarly, Wetmore (*71, p. 14*) pointed out that, although sugar refineries were located on each of three rivers in Utah at the outlets of which duck sickness had been prevalent, the seasonal discharge of these plants did not synchronize with the incidence of the disease. Mortality ceased while the mills were still in operation. These observations discount the likelihood of finding a direct and immedi-

ately acting causative factor in such discharges, but they do not explain away the possibility that such refuse, rich in organic matter, may later become a medium for toxin production under suitable conditions of alkalinity, temperature, and anaerobiosis (see p. 67).

Early in the history of the duck-sickness studies in Utah, it was alleged that organic pollution in the form of sewage from Salt Lake City was a causative factor, but this explanation also lost validity when the disease was later discovered at an increasing number of other points where there was no human pollution. There is, however, as in the case of discharges from sugar refineries, the possibility that sewage may increase locally the volume of toxin-producing media. More bacteriological research is necessary on these points.

LEAD POISONING

Although the idea of lead poisoning has not been seriously advanced as an explanation of true duck sickness, the two maladies have occurred simultaneously in the same area. At such times confusion may result in differentiating them, and birds apparently affected by duck sickness may in fact be suffering from lead poisoning. A judgment based merely on inability of the birds to fly or to walk is inadequate. A fuller diagnosis will leave no doubt as to the identity of the malady concerned. Points of difference between the two maladies were detected and defined by Wetmore in the course of his studies in Utah in 1914–16 (*72*). He found that birds with lead poisoning have good appetites and continue to feed even though the gizzard, by reason of its paralysis, fails to triturate the food, and that this often leads to a great distention of the proventriculus. In duck sickness a loss of appetite is characteristic. The percentage of recovery from lead poisoning is low, and improvement is greatly retarded, whereas in duck sickness there is a high percentage of recovery and sometimes this is almost spontaneous. Paralysis of the nictitating membrane is not a symptom of lead poisoning, though in duck sickness it is common. In lead poisoning, in addition to a brilliant green discoloration of the gizzard contents and a distention of the gall bladder, one may frequently find the telltale shot in the stomach itself.

GASES

The presence of methane, hydrogen sulphide, and other gases in duck-sickness areas has given rise to the belief, quite prevalent in some places, that duck sickness is produced by the inhalation of gaseous toxins. There is little basis for this belief other than the fact that these gases are present, and that in sufficient concentration they are toxic. The marked dissimilarity, however, between the symptoms of duck sickness and those resulting from the inhalation of these gases precludes serious consideration of the latter as a factor. As a check against a report that paralysis of the nictitating membrane, a characteristic symptom of duck sickness, may be induced by hydrogen sulphide, asphyxiation experiments on ducks were conducted, but in no case was paralysis of the nictitating membrane observed. Furthermore, as was to be expected, the whole syndrome of hydrogen-sulphide intoxication is so radically different from that of duck sickness that the two cannot be confused or considered to have anything pathologically in common.

BACTERIAL INFECTION

The concept of a bacterially produced duck disease dates back to the first investigations. Much of the early experimental work was devoted to attempts to produce or transmit the malady by one or another of the methods commonly employed in conveying bacterial infections. Every attempt of this kind, however, met with failure. Wetmore in 1918, related some of his experiences in the following words (*71, p. 13*):

> Healthy birds were confined with sick birds or were given grain treated freshly with feces taken from affected individuals. Some were fed forcibly on fragments of organs or the entire stomach and intestinal content of sick birds. The mucous lachrymal discharge in birds far gone was transmitted to the eyes of some. Intravenous and hypodermic transfusions of blood were made. All these experiments gave negative results.

Specimens sent to the Bureau of Animal Industry as early as 1914 were closely examined, but no diagnostic lesions were revealed. Numerous inoculations made from different organs of the sick birds, both on culture media and into experimental animals, failed to disclose any organism that might be regarded as the causative agent of the disease (*70, p. 5*).

Symptoms were carefully noted with respect to similarity to the syndrome of the avian bacterial diseases then prevalent. It was observed that to a certain degree avian cholera (caused by *Pasteurella avicida*) resembled duck sickness in that there was a general depression that might be mistaken for paralysis, a diarrhea, a pronounced thirst (prevalent in birds recovering from duck sickness), labored breathing, and, shortly before death, complete prostration. Discrepancies existed, however, in the above-normal temperature in avian cholera as against the uniformly subnormal temperature in duck sickness, and in the characteristic hemorrhagic areas present on the surface of the heart in avian cholera compared with the absence of the pronounced lesions in duck sickness. Furthermore, inability to transmit the disease by the means employed in the experimental work afforded added evidence against cholera as the cause. In fact it was the lack of infectiousness, the lowered body temperature, and absence of the characteristic post-mortem aspects of duck sickness that distinguished it from all other avian bacterial diseases generally known at the time.

Failure to demonstrate bacterial infection marked the efforts of H. Van Roekel in California in 1929 (*69*). Shaw, in 1930, stated: "Attempts to culture a causative organism from the tissues and intestinal tracts of the diseased birds gave negative results" (*62, p. 564*). Experiments aiming to reproduce the disease by placing birds in a suspected pathogenic environment also were inconclusive.

Despite failures in the earlier experiments and despite the fact that, throughout the years, thousands of sick and convalescing birds had been kept in intimate contact with healthy stock or with birds that had fully recovered without having transmitted the disease, the idea of some obscure bacterial trouble was not wholly discarded even when the investigation was resumed in 1927.

As late as the field season of 1930 a number of inoculation experiments involving the use of blood serum and brain emulsion of afflicted birds were conducted. Doses of 0.5 to 2.0 cc of the serum,

administered subcutaneously beneath the wing or intraperitoneally, failed to affect pintails and mallards; and injections of brain emulsion varying from 0.1 (injected directly into the cerebrum) to 0.5 cc (injected just anterior to the atlas vertebra) gave negative results with pintails. When it became evident later that the malady was caused by the toxin of a saprophyte, generated under conditions of decay outside the body, attempts to convey this noncontagious disease from bird to bird were discontinued.

PARASITES

The possibility of parasitic infestation also has been considered in attempts to solve the mystery of duck sickness. Coccidia found in the intestinal tract of ducks in the course of early examinations were looked upon with suspicion, but this was not substantiated by later studies. Limited study of parasitic nematodes at Great Salt Lake also failed to disclose positive evidence (*70, p. 5*). In later years observations in California militated further against the possibility of parasites being to blame. E. C. O'Roke, then conducting investigations for the Division of Fish and Game of the California Department of Natural Resources, came to the conclusion: "From the standpoint of intestinal parasites, the survey thus far has disclosed no apparent relationships existing between duck sickness and the presence of parasites" (*53, p. 294*).

Dr. Cram contributed substantially to the knowledge of parasites and their negative relationship to duck sickness in the course of a month's field research at the Klamath Falls laboratory in 1929 and in subsequent study of the material collected. Detailed observations were made on a total of 41 birds—including 18 pintails, 10 California gulls, 3 avocets, 2 western sandpipers, 2 Wilson's phalaropes, and 1 each of 6 other species. The report[4] shows that—

> At post mortem examination respiratory and digestive tracts and the body cavities were scrutinized for the presence of worm parasites, and smears were made from the respiratory, digestive, excretory, and circulatory systems for microscopical examination for protozoan parasites.
>
> Parasites were found to be present in a high percentage of the birds examined and often in heavy infestations. However, the fact that control, that is, unaffected, birds also showed parasites; that, in the cases in which a considerable number of sick birds of one species were available for examination, a few of the birds showed no parasites, although they showed the same symptoms as the parasitized birds; and, finally, the fact that the parasites found in the sick birds represented a wide variety of species, with no indication of the predominance of any one occurring throughout the various species of birds, all indicate that duck sickness is not correlated with parasitic infestation.

It was determined, however, that, in several instances, parasitism was of a degree that indicated definite injury to the bird host. These injuries included the formation of necrotic areas in the glandular and corneous walls of the proventriculus and the gizzard, respectively, and the formation of tumors at the junction of these organs; inflammation and thickening of the wall of the small intestine were also noted. Although no experiments were made in this investigation to determine the effect of lesions in the alimentary tract on the

[4] CRAM, E. B. REPORT ON AN INVESTIGATION OF THE RELATION OF PARASITES TO "DUCK SICKNESS" IN THE KLAMATH FALLS REGION, 1929. (Manuscript on file in Bureau of Biological Survey.)

assimilation of *botulinum* toxin, Graham and Boughton (*26, p. 23*) have referred to the possibilities in this direction. By the puncturing of the stomach or intestinal walls, parasites may play a secondary or predisposing part in duck sickness.

At Lake Malheur, Lake Newell, and at other points, observers have noted, in the course of duck-sickness outbreaks, a prevalence of leeches, these clinging to and even clogging the nostrils and trachea of disabled birds. To one unfamiliar with the course of events leading to this condition, leeches might appear to be the causative factor in the birds' disability. Although the annelids may add greatly to the discomfort of such helpless birds and probably hasten the death of many, they cannot be looked upon as the primary cause of the disease. The presence of these parasites is a result, not the cause of the malady, since the birds, hopelessly paralyzed and often prostrate, cannot prevent the leeches attaching themselves. Total absence of leeches in typical outbreaks of duck sickness at other points militates against the possibility of their being causative factors.

Mention may be made of the observed presence of dead snails surrounding the carcasses of birds that had died of duck sickness. Although the phenomenon was not noted about Klamath Falls, it was reported common at Lake Malheur in the outbreak of 1925, and at that time there was a popular surmise that snails were playing the role of intermediate host for some parasite. While the present understanding of duck sickness completely eliminates this concept, the presence of the snails nevertheless may have had peculiar significance. It is possible that these mollusks, dying from a lack of oxygen or other cause, may have served to produce the toxic material later ingested by ducks and shore birds. In this manner snails may serve as secondary causative factors of the disease, though not intermediate parasitic hosts.

DIETARY DEFICIENCY

That duck sickness is a nutritional malady has been contended by some who have endeavored to interpret the symptoms. L. Alva Lewis, a proponent of the idea, asserted duck sickness among waterfowl to be the direct result of a diet characterized by a marked excess of wheat and other grain, with a corresponding scarcity of green foods.[5] The resultant excess of vitamin B and deficiency of vitamins A and C were looked upon as the causative factor of the paralysis and other symptoms. It was held that the harvesting of the grain crop late in summer and a coincident decrease in the supply of green food were responsible for the dietary upset.

Polyneuritis, a nutritional disease among domestic fowl subsisting on a diet deficient in the water-soluble vitamin B, may be mentioned, not as being involved in any disorder of wild birds, but because of the similarity of some of its symptoms to those of duck sickness. There is a subnormal temperature, as in botulism; a progressive paralysis, the first manifestation of which is an unsteadiness on the feet, with a tendency to lean forward onto the toes (p. 48); a leg weakness, revealed at the tibiotarsal joint and causing the bird to drop suddenly when squatting; and, at the end, utter prostration.

[5] LEWIS, L. A. NEUROTIC DYSTROPHY AMONG DUCKS OF THE FAR WEST. 1927. (Manuscript report submitted to Bureau of Biological Survey.)

Despite the marked similarity of some of the symptoms of duck sickness and those of a dietary upset, the nutritional-disease theory loses validity when certain attendant factors are considered. Duck sickness has frequently appeared when green food in the form of *Potamogeton* and other submerged vegetation was plentiful. It also has ceased when grain still was being eaten and no additional supplies of green food were available. The malady affects alike such widely divergent forms as the highly vegetarian ducks, the insectivorous shore birds, and the omnivorous magpie. Moreover, a high percentage of the sick birds recover without correction of any suspected deficiencies in diet. In fact, the thousands of birds that have been salvaged (pl. 6, *B*) in years past have in the main been fed on rations more deficient in vitamins A and C than was their lot under field conditions. Furthermore, to test the validity of the nutritional-disease theory, Sperry in 1927 demonstrated that mallards and pintails, sick with the disease, effected recovery as quickly and as completely when fed on an exclusive diet of wheat (for a week or 10 days) as when given a mixed ration.[6] In the normal process of recovery, the desire for food of any kind is one of the last of the impaired impulses to be regained by birds affected with duck sickness, and for this reason its character can play, at best, a minor and delayed function in the process of recuperation.

TOXIC ALGAE

The hypothesis that algae may be involved as causative factors in duck sickness acquired a degree of plausibility from observations made in the course of the outbreak at Lake Newell, in 1925 (*51*). J. A. Munro noted that a marked change had taken place in the character of the submerged flora since his earlier observations in 1921, and that a luxuriant stand of *Potamogeton* had to a large extent been displaced by masses of blue-green algae, which decomposed later in the summer and accumulated in vile-smelling masses in shallow and sheltered portions of the lake. Such a condition of decay did not exist in the following year, when the water level had lowered, and the malady did not reappear.

The presence of extensive algal growth of both the blue-green (Myxophyceae) and the green algae (Chlorophyceae) has been noted at other points coincidentally with outbreaks of duck sickness. In 1928 a prevalence of what were apparently blue-green algae was observed at the American Falls Reservoir, Power County, Idaho, during a period when duck sickness prevailed. In the following year a similar condition was noted at Long Lake, Kidder County, N.Dak., during the peak of an outbreak of the disease. In the Klamath Falls district, filamentous green algae (Chlorophyceae) have dominated the submerged flora of some of the bodies of water where the sickness has occurred. Late in summer these beds, rising to the surface, cover extensive areas, and by their greenish yellow coloration add much to the brilliance of the scene (pl. 2, *C*).

In August 1929, Marsh visited Tule Lake, near the seasonal peak of the outbreak of duck sickness, to learn what part algae, particu-

[6] SPERRY, C. C. REPORT ON DUCK SICKNESS IN SOUTHERN OREGON AND NORTHERN CALIFORNIA IN THE SUMMER OF 1927. 1927. (Manuscript in files of Bureau of Biological Survey.)

larly those of the genus *Anabaena*, known to be toxic at times, may play in duck sickness. The center of the malady was on overflowed wheatland, at the northwest corner of the lake, and in that area the suspected blue-green algae were scarce. From observations made it was concluded that the possibility of algal intoxication was remote.

Algae may, however, play a secondary role in the incidence of the disease. These beds are the feeding grounds of many of the smaller shore birds (pp. 38–39). There the western and least sandpipers obtain the hydrophilid larvae, which, after death, may harbor the toxin of botulism (p. 58). It is in these tangled masses of algae also that many of the afflicted birds become imprisoned and lie exposed to the heat of the midday sun. There is the possibility (which at present has no proof) that the masses of algae cast up on mud flats may themselves serve, under suitable conditions, as incubating media for the bacteria of duck sickness. That they harbor in their skeins many minute organisms that may contribute to toxin production there is little doubt, and the impairment of water movement by dense algal mats tends to intensify conditions of decay and to prevent the dispersal or dilution of preformed toxin.

ALKALI TOXICITY[7]

The concept of toxic alkali as the causative factor of duck sickness was generally accepted as the result of experimental work conducted prior to 1918 by Wetmore, mainly in the highly saline environment of Great Salt Lake (*70, 71*). Although toxic alkali was looked upon with suspicion by Wetmore as early as 1915 it was not until the completion of his work that he definitely stated:

> By actual experiment it has been found that the duck sickness may be caused by the chlorides of calcium and magnesium. Experiments have indicated that other salts may be incriminated in Utah and elsewhere, but this statement is made with reserve, as it has not yet been definitely established (*71, p. 15*).

It is apparent from the foregoing that Wetmore felt that he had reproduced the syndrome of duck sickness in experimental birds by feeding them on suspected salts. Many other highly suggestive factors contributed to the conviction, quite generally entertained during the subsequent decade, that toxic alkali was to blame. In the first place duck sickness was, in a general way, associated with alkaline areas, and the outbreaks occurring in the San Joaquin Valley of California were looked upon as paralleling, in an etiological way, the malady at Great Salt Lake. The disease appeared to synchronize with circumstances that could be interpreted either as an increase in alkali or a more favorable opportunity for birds to ingest toxic salts. In addition there was suggestive evidence that a high percentage of sick birds transferred from an alkaline environment to one where they had access to fresh water recovered within a few days.

A number of other factors militating against an infectious bacterial explanation of the malady lent credence to the alkali theory in a negative way. Among these was the fact that the disease numbered among its victims many species of birds having a diversity of food habits and life histories, a condition ordinarily not encountered in infectious diseases and parasitism, yet strongly indicative of some

[7] The word "alkali" as used in this bulletin, refers to the combination of various salts, usually alkaline in reaction, found in western waters, muds, and soils.

widespread intoxication. Furthermore, the affected birds usually were in good flesh and, with the exception of a certain congestion of the arteries of the intestine, considered to be due to the irritant action of alkali, no characteristic lesions were evident.

Although as time went on, there were certain misgivings as to the adequacy of the alkali theory, it had in its favor a measure of experimental proof and a degree of plausibility possessed by no other single idea. When circumstances finally led to a resumption of investigative work by the Biological Survey (p. 2) the theory of alkali toxicity became a primary one to be proved or disproved in the light of new conditions and further direct experimental work.

In the analysis that follows, much of which deals with evidence against the postulate that duck sickness is caused by the toxicity of certain salts, full cognizance has been taken of the fact that some natural salts are toxic and under certain conditions may result in avian mortality.

CHARACTER OF WATERS

An analysis of alkaline waters with reference to duck sickness is presented in table 1, which shows in the form of percentages of the whole, the proportions of the bases and acid radicals of the saline components of 10 samples of water and 1 of mud extract obtained, with one exception, at the scenes of typical duck sickness. The single exception was that from Owens Lake, Calif., where the mortality of early days was at one time considered to be duck sickness, but on which later developments have thrown some doubt (p. 25). It is evident that even in a single area there may be considerable variation in the character and concentration of salines in different samples of water collected. For instance, at an irrigation sump south of Klamath Falls, total salt concentrations were recorded of from 1,700 to more than 200,000 parts per million, and variations in the chlorine radical from 1.52 to 7.95 percent. The analyses here discussed have been selected, however, as representative.

TABLE 1.—*Analyses of samples of western alkaline waters taken chiefly at scenes of typical duck sickness*

[The proportions of the various basic and acid radicals rather than those of hypothetical salts have been used in this table as a means of rational comparison. The figures indicate percentages of total anhydrous, inorganic solids. The composition of these waters in relation to the prevalence of duck sickness is discussed on pp. 14 to 17]

Sample	State	Percentages of component parts										Total salinity (p. p. m.)
		Cl	SO_4	HCO_3	Na	K	Ca	Mg	SiO_2	Fe_2O_3-Al_2O_3	Miscellaneous	
A	Utah	32.30	8.16	[1] 21.53	[2] 29.54	.....	10.12	4.76	.....	2.53	.....	637
B	....do	55.53	7.34	.23	32.56	.....	.97	8.15	.....	.....	0.22	102,658
C	....do	34.03	5.11	33.38	10.55	.....	7.32	0.61	.....	.....	.....	(3)
D	Oregon	3.45	3.83	62.52	1.15	.....	15.70	4.60	8.13	2.32	.....	261
E	....do	4.55	7.61	[1] 44.03	24.17	5.58	5.58	4.13	2.89	.....	.83	484
F	North Dakota	1.07	25.32	38.06	26.02	.....	.06	2.51	.08	.....	[1] 7.08	2,710
G	South Dakota	1.77	46.29	23.74	17.51	.....	1.03	7.44	.....	.....	1.62	1,696
H	Oregon	6.58	60.39	2.70	23.94	.....	1.76	4.37	.....	.....	.17	38,326
I	....do	3.80	.28	70.17	17.57	.....	5.13	2.94	.....	.....	.11	1,053
J	California	4.01	9.80	58.92	18.54	.....	5.80	1.80	.....	.82	.12	847
K	....do	24.82	9.95	24.55	38.69	1.02	.02	.01	.14	.04	.78	213,700

[1] Normal carbonate.
[2] And potassium.
[3] Not computed; see description of sample.

The samples of water were obtained as follows:

A. Near mouth of Bear River at Corrine, Utah. Analysis received from Southern Pacific Railroad (*9, p. 158*).

B. Mouth of Weber River, Utah. Collected by Wetmore subsequent to the peak of duck-sickness outbreak. Examination by Bureau of Chemistry and Soils, United States Department of Agriculture.

C. Water soluble material from muds in sickness areas on the Bear River Delta. Collected by Wetmore. Examination by Bureau of Chemistry and Soils.

D. Mouth of Blitzen River, Lake Malheur, Oreg. Collected by Ray C. Steele while duck sickness was prevalent in August 1926. Examination by Bureau of Chemistry and Soils.

E. Lake Malheur, Oreg. (*9, p. 163*).

F. Long Lake, Kidder County, N.Dak. Collected in August 1917. Examination by Bureau of Chemistry, United States Department of Agriculture.

G. Lake Poinsett, Hamlin County, S.Dak. Collected September 12, 1929, by F. M. Uhler while an outbreak of duck sickness was on the wane. Examination by the Water and Beverage Section, Food, Drug, and Insecticide Administration, United States Department of Agriculture.

H. Shallow pool at Government sump, 10 miles south of Klamath Falls, Oreg. Collected by Sperry in 1927. Examination by the Food, Drug, and Insecticide Administrarion.

I. Flooded Field near Keno, Oreg. Collected by Sperry in 1927 during a duck sickness outbreak. Examination by the Food, Drug, and Insecticide Administration.

J. Lower Klamath Lake, Siskiyou County, Calif. Collected by Sperry during a limited duck sickness outbreak. Examination by the Food, Drug, and Insecticide Administration.

K. Owens Lake, Calif. (*9, p. 162*). Sample taken in August 1905. Analysis by C. H. Stone.

Despite the fact that all but one of these samples came from areas in which duck sickness was prevalent or has occurred, there is wide diversity in the proportions of the saline components. Even ions that theoretically may be combined to form the chlorides of calcium and magnesium, formerly considered causative factors of the disease, are by no means uniform or conspicuous in the several samples. Sample D, for instance, collected at Lake Malheur during a disastrous outbreak, though containing calcium in moderate proportion (15.7 percent), had little chlorine, and the total salinity (261 p.p.m.) was so low as to make the solution as a whole innocuous. Samples A and C (the latter a water extract from mud), collected at Great Salt Lake, revealed calcium, magnesium, and chlorine ions in fair proportions, yet even though these were combined to form chlorides of calcium and magnesium, they still would comprise a minor part of the saline complex, and could be considered toxic only in high concentrations. In all other samples these two chlorides formed even smaller proportions. In no case did calcium and magnesium chlorides dominate the saline content.

The other ions that in one form or another might be considered toxic to birds show great variation in the samples. The proportions of sodium, carbonate (bicarbonate), and sulphate ions as well as chlorine (previously discussed in combination with calcium and magnesium) fluctuate from a position of dominance to one of insignificance. There appears to be no salt or combination of ions that is a characteristic of the alkaline waters collected in these widely separated duck-sickness areas.

For a study of the alkaline waters of the Klamath Falls section, the analyses of 26 samples are available in addition to the 3 presented

in table 1. These were collected at Tule Lake, at the Government sump 10 miles south of Klamath Falls, and from various adjacent ponds, canals, or flooded fields.

Of the 26 samples, 13 were collected in duck-sickness areas, either just before, during, or just after an outbreak; the other 13 were collected under circumstances not intimately associated with the disease. Data concerning the proportions of the sodium, magnesium, calcium, and chlorine ions in these two groups are presented in table 2.

TABLE 2.—*Sodium, magnesium, calcium, and chlorine ions (expressed as percentages of total soluble salts) in samples of water from duck-sickness areas and from areas free of the disease, based on the analysis of 26 samples collected mainly in the vicinity of Klamath Falls, Oreg.*

Source of samples	Samples	Na	Mg	Ca	Cl
	Number	*Percent*	*Percent*	*Percent*	*Percent*
From duck-sickness areas	13	10.02	2.01	6.27	2.61
From areas free of sickness	13	17.93	4.99	3.81	4.39

It is apparent that none of these waters of the Klamath Falls section is dominated by the chlorine ion, as the average percentage of this element in the whole group of samples was only 3.5. The percentages of chlorine, sodium, and magnesium were greater in the waters from nonsickness areas, while the reverse was true of calcium. Such conditions might be construed as confirmatory of the alkali theory insofar as it applies to the toxicity of calcium chloride, but it is to be remembered that the differences with respect to the salts in the sickness and nonsickness areas are merely in degree. When it is realized that the whole matter is subject also to the highly variable factor of concentration, these differences in percentages in what are in fact minor elements in the saline complex do not appear pronounced enough to account for the presence, or the total absence, as the case may be, of an epizootic.

In this discussion of the character of salts in sickness and nonsickness areas it is fitting to call attention to the published findings of Shaw (*61*) in California, that soils from certain disease areas contained a relatively high percentage of nitrate ions, while comparable soils from nondisease areas revealed very little. This was interpreted as being highly suggestive of his contention that toxicity may be increased by the addition of nitrate ions to what otherwise might be more or less harmless saline combinations (*60*). At the time these ideas were advanced the whole matter was treated from a toxicological standpoint, and, although it was recognized that nitrates often are the end product of bacterial processes, the significance of this interpretation in relation to the real cause of duck sickness was not then appreciated.

In the work at Klamath Falls, effort was made to repeat Shaw's experimental work, which had been conducted largely in California. Couch gave considerable attention to this aspect of the case and made numerous tests for nitrites and nitrates in the muds of sickness and nonsickness areas.

In the determinations made during this investigation the results showed that the largest proportion of nitrates was obtained from a pond [Largent's] where there was no outbreak of the disease this year (1930) and smaller amounts occurred in areas where the disease was in active progress."[7a]

Appraisal of the character of waters in sickness and nonsickness areas has yielded little to confirm the earlier concept of alkali toxicity postulated by Wetmore and its variant advanced by Shaw. No direct synchrony between the character of alkali and the incidence of duck sickness was revealed except that brought out by Shaw, which is subject to more logical interpretation on bacteriological than toxicological grounds. There remained, however, the necessity of determining, approximately at least, what concentrations of salines commonly found in these waters, are actually toxic to waterfowl, and hence a potential danger.

TOXIC CONCENTRATIONS

Ingested in sufficient quantity, sodium chloride is toxic to birds. Experimental work has demonstrated that this salt, administered daily, at the rate of 4 g per kilogram of body weight, is close to the minimum lethal dose for a chicken weighing 3 to 5 pounds (*50*). It has been shown by Heller and Larwood (*34*) that Epsom salt (magnesium sulphate), a common constituent of western alkalis, has a retarding effect on growth of small mammals when used in drinking water in concentrations of 15,000 p.p.m., and that it is decidedly toxic in concentrations of 25,000 p.p.m. Calcium chloride is even more toxic, yet in weak solutions (2,000 p.p.m.) this salt was found harmless when used in drinking water of white rats (*49*). "The chlorine ion appears to be more toxic than either the carbonate or the sulphate ion. The calcium and magnesium ions are more harmful than the sodium ion" (*34*). Shaw (*60*) has demonstrated experimentally the toxicity to ducks of sodium chloride, magnesium chloride, and calcium chloride as well as certain mixtures of these and other salts.

In the course of this investigation it was demonstrated, through an experiment conducted in pens erected over a natural pool of alkaline water, that saline solutions of the character prevalent about Klamath Falls are highly toxic to several species of birds whenever the concentration is as great as 25,000 p.p.m., provided such solutions are the sole source of drinking water. In character the dissolved salts were largely sulphates with carbonates and bicarbonates in moderate proportions and chlorides forming a negligible part. Sodium was the dominant base. The birds subjected to such experimentation included gulls of two species, coots, and redhead and mallard ducks. The same saline solutions in concentrations of 5,000 or 2,000 p.p.m. appeared not to be harmful.

Seventeen other experiments were conducted to learn more of the character and concentration of alkali needed to produce intoxication among ducks using the solutions as drinking water. In eight of these the birds died apparently from the toxic effect of the dissolved salts. Death occurred in every completed experiment in

[7a] COUCH, JAMES F. REPORT OF INVESTIGATION OF SO-CALLED ALKALI POISONING OF DUCKS AT KLAMATH FALLS, OREG., IN 1930: RESULTS OF ANALYSES AND EXPERIMENTS. 1930. (Copy of manuscript on file in Bureau of Biological Survey.)

which the solution approached the salt concentration of ocean water (31,000 to 36,000 p.p.m.), and in one instance a concentration of 26,000 to 27,000 p.p.m. killed a bird that had been taking it only 2 days. In two experiments in which higher concentrations were used (80,000 and 200,000 p.p.m., respectively), the birds refused to drink, indicating that there is a point at which ducks may prefer to die of thirst rather than take the exceedingly toxic waters. On the other hand, solutions containing salts in a concentration of 3,353 p.p.m., half again as strong as the highest concentration recorded for the open water of Tule Lake, failed to affect birds drinking it for 7 days.

Knowing the minimum lethal concentration (for birds) of the saline solutions prevalent in the Klamath district (20,000 to 25,000 p.p.m.), it is possible to appraise conditions more definitely with respect to the likelihood of birds ingesting lethal doses under field conditions. The highest concentration of salts recorded for any water sample in the immediate vicinity of Tule Lake was 8,079 p.p.m. This sample was collected in a small depression adjacent to the lake at a time when the water table was slowly rising and absorbing the encrusted surface alkali.

Something of the harmlessness, even to birds afflicted with duck sickness, of saline solutions containing as much as 11,500 p.p.m. of dissolved salts, was learned at the Bear River (Utah) Migratory Bird Refuge during the summer of 1932. In that season 1,819 sick birds were gathered and placed in an enclosure where, for bathing and drinking purposes, they had access to a pool formed by an artesian well. This water contained sodium chloride in the concentration of 9,787 p.p.m. and other salts, including magnesium chloride, calcium carbonate, and magnesium carbonate, in quantities sufficient to bring the total salt content to the concentration mentioned. Despite the fact that this was the sole source of drinking water for these sick birds, and that they were given practically no individual attention, 54 percent recovered.

It would seem, therefore, that the postulate of alkali toxicity if applied to the Tule Lake basin must rest on the assumption that the birds were locating saline pools of a concentration greater than any revealed by this investigation, or else were obtaining toxic alkali at other points in the Klamath Falls district where such concentrates existed. Field observations on the birds themselves indicated the ducks did not habitually leave the Tule Lake basin during August and September, since within it were all the essentials for existence—water, food in the surrounding grainfields, and shelter and protection on the Federal bird refuge. However, at the Government sump, an irrigation basin south of Klamath Falls, solutions of salines far above the minimum lethal concentration were to be found in pools about the border. Under such conditions, which are comparable with those existing at many other of the more highly saline duck-sickness areas of the West, it is possible for waterfowl to come in contact with lethal quantities of toxic salts throughout the summer and early fall. Yet at the same time and place the relatively fresh water (3,000 p.p.m.) of the main sump supported a luxuriant stand of *Potamogeton pectinatus*, which served as a constant attraction to the waterfowl. If the theory of alkali intoxication is entertained under such conditions, it must be predicated upon the assumption that sufficient

toxic salines are ingested by birds on visits to the shore line, despite the fact that most of their feeding and drinking take place in the essentially wholesome water of the open sump.

Another consideration of paramount importance, but usually overlooked in attempts to explain duck sickness on the basis of alkali toxicity, is the reaction of birds to the taste of toxic saline concentrations. In every duck-sickness area visited there have been, within a short flying distance from any toxic alkaline pools that may have existed, ample supplies of suitable drinking water. At times the distance from one to the other was a matter of only a few yards, and if the birds possessed any marked aversion to the toxic waters it would be difficult under such circumstances to conceive of thousands ingesting lethal doses.

Mention has been made of the refusal of ducks to drink exceedingly high saline concentrations (80,000 and 200,000 p.p.m.), in experiments conducted in this study (p. 18). Others also have noted this fact. It remained to be demonstrated, however, whether concentrations of about minimum lethal strength would be refused by birds having equal access to wholesome water. An experiment was undertaken therefore, with a solution of natural alkali, composed mainly of sulphates, prepared in a strength of about 20,000 p.p.m. By trial this was found to be nontoxic to ducks using it as drinking water for a period of a week. When this was offered along with equally accessible fresh water, a marked aversion to the saline solution was revealed.

From the foregoing it may be stated (1) that solutions containing dissolved alkali of the Klamath Falls district in concentrations as great as 20,000 p.p.m. have been shown to be lethal to ducks when used as the sole source of drinking water; (2) that although such concentrations may be found in pools in the vicinity of certain duck-sickness areas, extensive outbreaks have occurred without their presence; and (3) that waterfowl have shown a sense of discrimination, even against nontoxic solutions of salts, that in all probability would lead them to shun toxic concentrations when fresh or relatively fresh water is close at hand.

LACK OF SYNCHRONISM BETWEEN DUCK SICKNESS AND SEASONAL FLUCTUATIONS IN ALKALI CONCENTRATION

Other factors being equal, it might be assumed, on the basis of the theory of alkali toxicity, that duck sickness would have a seasonal incidence conforming to the period of high concentrations of alkali. In general this is true, but a day-by-day study of conditions of alkalinity, before, during, and after an outbreak of the disease, fails to demonstrate a real synchrony between duck sickness and high concentrations of alkali.

The outbreak of duck sickness that occurred on overflowed land at the northeast corner of Tule Lake in 1929 began under a condition of slowly rising water, which had existed for some time previously. The gradually extending sheet of water was absorbing encrusted alkali, and as it crept forward it formed areas of relatively high saline water, although not in concentrations that could be considered toxic (p. 18). This process had started about the first of July and continued steadily until well into October. In the course of that

process sickness appeared about August 1 and disappeared about September 20.

The failure of duck sickness to appear at the same point when essentially similar saline conditions are re-created in different years was brought out in the course of field work at the Largent Pond, previously mentioned. Prior to 1928 the pond evaporated to dryness during summer, but in that year it was kept full throughout the season by a break in a nearby irrigation ditch. Duck sickness appeared and caused the death of almost an entire flock of domestic ducks frequenting the area. In 1929 the break in the dike was repaired and again the pond evaporated to dryness with no sickness in evidence. In 1930 this area was used as a site for experimental work. The depression was flooded and a constant water level was maintained, approximating that prevailing in 1928 when sickness occurred. Alkali conditions also appeared to be similar. Despite the fact that alkali toxicity was demonstrated with birds confined in pens on the shore line of this pond (p. 17), and notwithstanding the presence of both domestic and wild birds on the pond throughout the season, true duck sickness did not appear.

Besides these and many other instances of lack of direct synchrony between duck sickness and high concentrations of saline solutions it has been Kalmbach's frequent observation, while engaged in recording water levels, alkalinity, and other factors having a possible bearing on the cause of the disease, that the seasonal increase in alkalinity is gradual. Duck sickness, on the other hand, may become a pronounced epizootic almost spontaneously. With the termination of seasonal outbreaks, however, a decrease in alkalinity may, with sounder logic, be interpreted as an influencing factor, since the disease invariably subsides with the coming of fall rains or with a pronounced increase in water depths, either of which would tend to dilute saline waters.

SYMPTOMS OF ALKALI POISONING AND DUCK SICKNESS COMPARED

Differences noted between the clinical as well as post-mortem aspects of cases of duck sickness and of alkali poisoning, experimentally produced, have aided in distinguishing the two maladies. Not a single typical case of either acute or chronic duck sickness was produced in more than 50 individual experiments involving the administration of alkali or alkali solutions. In this connection mention again may be made of the toxicological studies of Shaw, who stated in his final published contribution on the subject that, although he felt that some symptoms comparable to those of duck sickness had resulted from the feeding of alkali, he "was not satisfied that the identical picture of the disease has been reproduced" (*62, p. 567*).

In view of their negative nature merely a summary of these experiments will be presented. They may be divided into two groups: One of 17, in which the salts were conveyed in solution either as drinking water or as doses administered through a pipette; and another of 34, in which dry salts, enclosed in capsules, were force-fed. The major portion of the solutions offered as drinking water were made from encrusted alkali found in the Klamath Falls district, and whenever the quantity of dissolved salts approached or exceeded 30,000 parts per million, fatal results ensued. In the feeding of dry

salts, including quantities of natural alkali, only one bird (affected by complications of unknown character) died. In these experiments many synthetic combinations of alkali also were prepared, including the local dominant salts as well as those suspected at Great Salt Lake. Both calcium chloride and magnesium chloride, singly and in combination with each other and with other salts, were fed in doses of 6 to 8 g without ill effects. Single doses of similar size were given, involving natural and synthetic alkali to which were added quantities of nitrates, with a view to increasing the toxicity. In none of these were symptoms characteristic of duck sickness produced.

In comparing the syndrome of alkali intoxication and duck sickness, one is confronted, on the one hand, with variations in the character of alkali as found in nature and, on the other, with differences arising from the severity of the attack in duck sickness, which may vary from a slight impairment of gait or flight to a condition of complete prostration. That certain physiological reactions arising from each are so similar as to be easily misinterpreted adds to the difficulties of comparison. The following presentation of analogous reactions sets forth some of the more obvious symptomatic and pathological differences and similarities of alkali poisoning and duck sickness as observed in this study:

Alkali poisoning	*Duck sickness*
1. A general depression occurs in lethal cases, which at times takes on the aspects of a paralysis. The power of sustained flight is lost; the legs become weak, and an unsteady or stumbling gait results; eventually the victim can no longer stand, whereupon death follows, often spontaneously.	1. A paralytic condition, affecting in turn the muscles of wings, legs, and neck, is characteristic, but except in extremely acute cases death follows a gradually increasing lethargy. Complete prostration is typical for a variable period previous to death, which usually takes place without struggle.
2. Respiration may be weakened and its rapidity slackened (a condition appearing immediately prior to death), but there is no well-defined air hunger during the middle stage of disability.	2. The progressive paralysis affects muscles controlling pulmonary movements. This manifests itself as an air hunger, giving rise to a gasping effort at every breath. It is a frequent and fairly early symptom in most birds affected, and is particularly pronounced in gulls.
3. With the increasing impairment of voluntary and involuntary faculties the nictitating membrane of the eye is sometimes affected. This usually results simply in a slowing up of its functions but at times the membrane may become immobile. The winking of the eye may be a slow, complete, but infrequent process.	3. Paralysis of the nictitating membrane is a frequent but not a pathognomonic symptom. Unlike its occurrence in alkali poisoning, it is not confined to the final stages. In lethal cases it appears early; in sublethal cases it usually synchronizes with the peak of the syndrome, or may even be absent entirely. A much more constant accompaniment of duck sickness than of alkali poisoning.
4. Fluid discharges from the nostrils as well as from the eyes have been noted in experimental birds subjected to alkali treatment, but in no instance have the discharges observed been of a character or in profusion sufficient to clog the glottis or seal the eyelids.	4. Profuse discharges from eyes and nostrils are frequent and may thicken into cheesy masses that clog the glottis, block the trachea, or harden and seal the eyelids. According to Wetmore much of this has its origin in enlarged Harderian glands at the anterior corner of the eyes.

Alkali poisoning	*Duck sickness*
5. A diarrhea is frequent, a natural result of the purgative effect of the salt ingested.	5. A greenish diarrhea is common, terminating with the discharge of increasing quantities of white renal matter, the subsequent hardening of which may completely obstruct the vent, with a resultant distention of the cloaca.
6. A lowered body temperature with a weakened, less rapid, or irregular heart action is common in the later stages.	6. A subnormal body temperature is likewise characteristic. Even in sublethal cases a temperature of less than 100° F. has been noted in ducks.
7. The alimentary tract almost invariably reveals evidence of severe irritation. There have been observed profuse mucous discharges from the esophagus and the glandular portion of the proventriculus, a sloughing of the edges of the gizzard lining, and a quite constant congestion of the blood vessels of the intestine, particularly those of the duodenal loop.	7. Post-mortem examinations in the Klamath area showed no gross lesions in the intestinal tract comparable with those arising from the irritant action of salts. Occasional congestion of the blood vessels, a hardening and contraction of the walls of the smaller intestine, straw-colored contents, and a distention of the cloaca anterior to an obstructed vent are the only post-mortem manifestations that occur with any degree of frequency in the intestines.

Of the distinguishable differences thus revealed in the syndromes and post-mortem conditions of alkali intoxication and duck sickness, those outstanding are the spontaneity of death and the presence of very evident lesions, in alkali intoxication; and, in duck sickness, the presence of an air hunger, the frequent paralysis of the nictitating membrane, and the lack of pronounced lesions. There are other more or less subtle and less clearly defined points of difference which, though they cannot be described adequately or appraised, can be detected by the experimenter and field observer after many contacts.

In summarizing the subject of alkali toxicity and its possible relationship to duck sickness it may be pointed out (1) that appraisal of the chemical composition of waters in sickness and nonsickness areas about Klamath Falls has yielded little to strengthen the alkali theory; (2) that, although concentrations of dissolved salts as low as 26,000 p.p.m. are lethal when such solutions are used as the sole source of drinking water, pronounced outbreaks of duck sickness have occurred in areas where such lethal concentrations did not exist; (3) that healthy experimental birds have shown an aversion even to nontoxic or mildly toxic concentrations of salts, and hence would be inclined to avoid highly toxic solutions where fresh water is equally available; (4) that there often is a lack of synchrony between the incidence of duck sickness and the seasonal period of high alkaline concentrations; and lastly, (5) that there are marked differences in the symptomatology and pathology of the two ailments.

OTHER STUDIES

The earliest contact of the Biological Survey with the investigative aspects of duck sickness occurred in May 1911, when Kalmbach, in company with officials of the New State Gun Club, inspected the area at the mouth of the Jordan River, Utah. Evidences of the great mortality of the previous year were still present in the many hundreds of disintegrated bodies of ducks and shore birds. The first

signs of the season's outbreak of duck sickness also were then appearing, a few affected birds being encountered. Two of these, a shoveler and a widgeon, were transported to the laboratory of the Bureau of Entomology, at Murray, Utah, where, placed in a bath tub, both recovered after 4 days and were released.

In August 1913, S. E. Piper, of the Biological Survey, made an examination of conditions at Great Salt Lake; and in the same year Frank C. Clarke, detailed by the California Fish and Game Commission and working in cooperation with the University of California, made an investigation and conducted experimental work at Tulare Lake, Kings County; and Buena Vista Lake, Kern County, Calif. (*7*) A report on Clarke's early work (*8*) indicated, as was noted at Great Salt Lake, that a high percentage of the afflicted birds recovered when placed in cages containing fresh water, but no definite causative factor was demonstrated.

In July 1914, Wetmore began an investigation that continued through the two following seasons. In the first year he made field studies at a number of points about Great Salt Lake, particularly in the marshes at the mouths of the Jordan, Weber, and Bear Rivers. Late in the season he visited Tulare and Owens Lakes. At the conclusion of the first year's work he prepared a preliminary report, which was published in May 1915 (*70*). This covered such aspects of the problem as its history and the theories as to cause and nature of the trouble, and led to the conclusion that the mortality was due to an alkaline poison, the exact nature of which still remained to be determined.

In 1915, he continued his studies and, as an aid, established a small field laboratory on the banks of the Bear River at the northern end of Great Salt Lake. Operations were continued there until the end of October with the exception of a short period of investigation at Lake Bowdoin near Malta, Mont., where an outbreak of duck sickness had occurred earlier in the season.

In 1916, the last year of Wetmore's studies at Great Salt Lake, field work began on May 15 and terminated on October 25. The final conclusions coincided with those published in the preliminary report (*70*), though certain salts of alkali, particularly the chlorides of calcium and magnesium, were then definitely incriminated. The report on the entire study at Great Salt Lake was published on June 21, 1918 (*71*).

After the publication of Wetmore's findings no further extensive research work in duck sickness was undertaken for nearly a decade. Although articles of a nontechnical character appeared from time to time in periodicals devoted to wild life, it was not until 1927 that the malady was again given serious study. Early in that year E. W. Nelson, then Chief of the Biological Survey, published the results of a general field survey of conditions existing in 1926 (*52*).

In that same year the California Fish and Game Commission in cooperation with the University of California undertook further study of duck sickness. Toxicological and pathological studies were conducted at the laboratories of the Hooper Foundation for Medical Research, with K. F. Meyer, director of the foundation, supervising. At the same time parasitological studies were conducted in the Zoological Department of the University of California, under the di-

rection of C. A. Kofoid (*44*). At the outset R. J. Irvine carried out chemical research, but later the purely toxicological and pharmacological aspects of the problem were handled by Paul A. Shaw, of the Hooper Foundation, who published several papers (*59*, *60*, *61*, *62*). Parasitological studies were started in November 1927, by O'Roke (*53*), who published his results in the following year. Pathological investigations were begun in June 1928 by Van Roekel, and a brief paper outlining his field of endeavor appeared in January 1929 (*69*), but at that time no additional light was shed on the nature of the malady. Field work connected with these studies was conducted at Tule Lake, and at points in the San Joaquin Valley.

In a review of the literature on duck sickness, mention should be made of three more recent contributions by M. Hobmaier, (*38*, *39*, *40*), who joined the staff of the Hooper Foundation for Medical Research in September 1930. In the following month Kalmbach had opportunity to reveal to Dr. Hobmaier the progress made at the field laboratory at Klamath Falls, accompany him on his initial inspection of the Tule Lake district of infection, and present him with culture material, from which he later isolated *Clostridium botulinum*, type C, thereby corroborating the earlier findings of Giltner and Couch (*25*, *p. 660*).

In his last contribution to the subject Hobmaier (*40*) presented a mass of significant and valuable data and adequately demonstrated duck sickness to be botulism by (1) the similarity of epidemiology and clinical picture, (2) the isolation of the causative organism, and (3) the demonstration of toxin in media in the field, as well as (4) in the blood stream of afflicted birds.

More recently the contribution of Gunnison and Coleman (*29*) to the fundamental bacteriology of the duck-sickness variant of type C botulism has appeared and supplied research workers with important descriptive facts not previously determined for this type.

HISTORY OF THE MORTALITY[8]

It is of importance in the consideration of a malady now looked upon as an outstanding menace to western waterfowl to note its history. Has this disease always been a factor in curbing the number of North American waterfowl, or is it a disaster of recent origin? If evidence points to its presence in early days, have the consequences always been as severe as they are in current times, when the mortality in some seasons is appalling? These are questions the answers to which not only have a direct bearing on a proper understanding of the problem as it is presented today but also are of importance in any attempted appraisal of what the future may have in store.[9]

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[8] The historical aspects of duck sickness here discussed concern the manifestation of this disease among wild birds; no attempt is made at this point to deal with the same or related maladies among domestic poultry or livestock. In the presentation of data concerning the history of duck sickness about Great Salt Lake, use has been made of the evidence compiled by Wetmore (*71*), to whom acknowledgment is gratefully made.

[9] The evidence on which early accounts of the malady are correlated with our present concept of duck sickness rests largely on the basis of similarity of symptoms and certain associated etiological factors. In a few of the localities in which the disease had been recorded, recent bacteriological determinations, by demonstrating the presence of the causative organism, have strengthened the supposition that early outbreaks were identical with those of today. There is always the likelihood, however, that other factors, including even direct toxicity of alkali, might have played a part in some of the early outbreaks. Consequently, no records about which there is a reasonable doubt are here included or, if mentioned, they are presented with appropriate explanation.

PERIOD PRIOR TO 1910

In discussing conditions at Great Salt Lake with respect to early records of the malady; Wetmore stated that—

> several hunters and guides who have shot on these marshes for many hunting seasons have recalled that sick and dead birds were found at an early day. Twenty-five years ago [1893] the hunting season began on September 1, and . . . It was not unusual to find many dead ducks on this date . . . Some of the early settlers in this region have reported sick birds at earlier times (*71, p. 2*).

A. K. Fisher, of the Biological Survey, reported finding many dead eared grebes and shovelers at Owens Lake in June 1891 under conditions that led him to believe their death may have been caused either by toxic alkaline salts, which were present in high concentrations, or by futile efforts to find suitable food in the highly saline water (*21, p. 12–13*). Nelson also reported large numbers of dead grebes in an exceedingly emaciated condition on the same body of water in December 1890 (*21, p. 13.*) A still earlier reference to bird mortality at Owens Lake, by Loew (*43, p. 190*), is to be found in Wheeler's report of 1876 on western geographical surveys, in which the statement is made that—

> notwithstanding the alkalinity of Owens Lake, numerous ducks are occasionally seen swimming on it. The great numbers of dead ducks and other aquatic birds seen here and there on shore seem to indicate that they tried to satisfy their thirst with this water.

In 1914 Wetmore visited Owens Lake, found many dead eared grebes and a few other birds, and stated, "apparently the cause of death was similar to that producing the duck sickness in Utah" (*71, p. 5*). In the absence, however, of definite bacteriological evidence of duck sickness at Owens Lake the record of its occurrence there is open to question. Several factors contribute to this element of doubt. One is that excessive mortality from this disease seldom occurs as early as June, the time of Fisher's observations; another, that general emaciation, as reported by Nelson, is not a common pathological condition in avian botulism; and, lastly, the high concentration of sodium carbonate in the waters of Owens Lake in all probability would make conditions highly unfavorable if not impossible for toxin production by the causative organism of duck sickness (p. 65).

Much of the literature of early exploring expeditions in the West has been searched with the hope of finding records of bird mortality that might be indicative of duck sickness. Among these records were the reports of military, engineering, and railroad survey parties and of geological explorations, and the writings of ornithologists who accompanied these expeditions. Ridgway (*55*) and Henshaw (*35*), both ornithologists, visited Great Salt Lake, the former in 1869 and the latter a few years after, but neither made comment on any extensive bird mortality.

Previously various expeditions had traversed the Great Basin and visited the shores of Great Salt Lake or the valley of Bear River to the north. These included the Ashley-Smith Explorations (1822–29), those of B. L. E. Bonneville (1833), and R. B. Sage (early forties), J. C. Frémont (1843), Howard Stansbury (1849–50), J. H. Simpson (1859), and others. These early explorers, all keen ob-

servers of wild life, made frequent mention of the abundance of birds, yet so far as the present reviewer can discern, no mention is made by any of an avian mortality that might be construed as an epizootic of duck sickness. During September 1843 Frémont (*22*) explored the Bear River Marshes and on September 9 traveled down the Weber River in a rubber boat, passed through the marshes at its mouth, and visited one of the islands in Great Salt Lake. From the standpoint of locality and time of year the opportunity of his noting duck sickness, had it been present during that season, was most favorable. Stansbury (*64*) explored the entire boundary of Great Salt Lake and also visited the Bear River and Weber River sections, yet he makes no mention of dead birds.

There are a number of later reports (previous, however, to 1880) concerning the central valleys of California, but in none was found reference to excessive bird mortality. Derby's Report of the Tulare Valley, 1852 (*12*), fails to mention any bird malady in a district in which endless thousands died in later years. In more recent times, what appears to be further negative evidence of duck sickness is found in reports of the Agricultural Experiment Station of the University of California covering the period from 1879 to 1889.

Particularly interesting is the recorded history of variations in the levels and alkalinity of the waters of the upper San Joaquin Valley with relation to animal life. Although the subject was studied primarily from an agricultural standpoint, Hilgard (*37*) in 1889 took note of the progressive destruction of mollusks and fish. He gave attention to the decrease in the fishing industry in the Kern and Tulare Basins in the 1879–89 decade, but despite close observation of wild life during that period, he noted no bird mortality. Wetmore stated that sick ducks had been observed in the Tulare Lake Basin for more than 20 years, which, it appears, would place the incipience of duck sickness as an epizootic there at some point in the nineties (*71, p. 5*). This antedates by 10 or more years the origin of the trouble in that vicinity as reported by the California Fish and Game Commission (*7*), which considered the year 1908 or 1907 as the first year of noticeable mortality.

Only one of the numerous outbreaks of suspected duck sickness of which there is record, reported from localities other than the vicinity of Great Salt Lake or the San Joaquin Valley, Calif., occurred prior to 1910. This one, of somewhat doubtful identity, appeared among geese at Bitter Lake, 25 miles northwest of Watertown, S.Dak., late in the fall of 1898.

It would seem, therefore, from the review of pertinent literature, that the earliest records of true duck sickness that may be pointed to with reasonable assurance are those mentioned by Wetmore for the early nineties at Great Salt Lake (*71, p. 2*).

PERIOD 1910–19

Waterfowl mortality in 1910 served for the first time to call national attention to the menace of duck sickness. It appeared at a number of points, but the outbreak at Great Salt Lake during that season was the most severe in any single area of infection. Waterfowl and shore birds died in such numbers that private clubs refused to open their grounds to hunting. In California during the

same season thousands of birds died in the Soleta, Goose, Buena Vista, and Tulare Lake sections of Kern and Kings Counties, in waters that are no longer existent, and from Saskatchewan, Canada, came reports of the death of "untold thousands" in the vicinity of El Fros, about 100 miles north of the United States. A short time later (1912–13) what appears to have been duck sickness was reported from the vicinity of Rice and Goose Lakes, west and southwest of Saskatoon. This locality still remains, so far as known, the most northerly for duck sickness on the North American Continent, a point fully 200 miles north of the Canadian border, at 52° north latitude.

In fluctuating but generally in decreasing intensity, duck sickness continued to take a toll throughout the decade 1910–1919. The year 1913 was one of considerable mortality in the upper San Joaquin Valley, and from Saskatchewan in the same year there came reports of renewed trouble near El Fros. At Great Salt Lake the year 1911 witnessed an improvement over the disastrous conditions of 1910 but in 1912 and 1913 excessive mortality again occurred. Conditions in 1914, when the Biological Survey began its initial study of duck sickness in Utah, were the best there since 1910. In 1915 extremely low water drove most of the waterfowl out of the infected area about the lake, with a resultant saving of many; but in the following season, after a return to nearly normal water conditions, duck sickness recurred in moderate intensity. During the remainder of the decade (1917–19) the disease occurred each year, but the losses were relatively small.

In 1915 Wetmore observed the disease at Lake Bowdoin, near Malta, Mont.; he also reported it in 1916 and 1917 at Baca Lake, 35 miles south of Lake Malheur, Oreg. (*71, p. 5–6*). Stanley G. Jewett and W. L. Finley observed duck sickness for the first time at Lake Malheur itself in 1917, and in the following year a few birds, afflicted apparently with duck sickness, were found in the vicinity of Lower Klamath Lake on the Oregon-California line while this body of water was evaporating to dryness. Burnie Maurek, game and fish commissioner of North Dakota, reported sick ducks at an alkaline lake in southeastern Kidder County, about 1915. In 1917 duck sickness was reported from the Salton Sea in southern California. A moderate outbreak occurred among the ducks in the Marysville Butte section of the Sacramento Valley, Calif., in 1918, and soon after there was added to the list of localities Dodson Lake, in the Warner Valley of south-central Oregon. During the latter half of this decade (1915–19) duck sickness, though appearing in localities not previously reported, nowhere assumed the serious proportions it had attained at Great Salt Lake and in the San Joaquin Valley in preceding years.

PERIOD 1920–32

Since 1920 the history of duck sickness has been characterized by a notable increase in the number of points of outbreak. Although part of this apparent spread results from the closer attention being paid to the welfare of wild life and the fact that duck sickness has become a matter of more common knowledge among sportsmen, it is evident that the disease appears today in epizootic intensity in many more localities than in 1920. It is still present, though in

fluctuating intensity, at most of the earlier recorded localities. At Great Salt Lake the year 1921 witnessed the greatest mortality since the outbreak of 1910; a decrease followed during subsequent years, and in 1924 additional improvement resulted through energetic frightening measures employed in driving the birds out of infected areas. Moderate mortality occurred in following years until in 1929, when, in August, there was a short-lived but severe outbreak at the mouth of Bear River. Frightening measures applied during the summer of 1930 curtailed what might have resulted in an epizootic of considerable extent, and in 1931, the disease was extremely localized. In 1932, however, the malady reappeared in its most violent form about the Bear River Bay, and on the south shore of Willard Spur the dead lay in numbers that recalled the catastrophe of 1910 about Great Salt Lake (p. 26).

What appears to have been true duck sickness among waterfowl on Utah Lake, 50 miles south of Great Salt Lake, was reported by Clarence Cottam, of the Biological Survey, as occurring in 1927 and 1928. Reports current at the time were that the disease had been noted several years previously.

In the upper San Joaquin Valley duck sickness was prevalent in varying intensity throughout the period, but on the whole the mortality was much less than during the previous decade. New spots of infection appeared, however, under peculiar etiological conditions on the artificially flooded and baited grounds of certain duck clubs. In 1927 there was great mortality in the vicinity of Buena Vista Lake, and in 1931 there was an outbreak of considerable intensity on the south shore of the Salton Sea at a point where the inflowing waters of the New and Alamo Rivers formed mud flats. In that year a few ducks died on the grounds of hunting clubs in the vicinity of Colusa, in the Sacramento Valley, under conditions strongly suggestive of duck sickness.

Early in this period the bed of Tule Lake was added to the list of known infected areas, later the scene of much of the field work of the present study. Although many waterfowl perished there during the first year that attention was attracted to the malady (1922), severe mortality occurred in 1925 and, to a less extent, in 1926[10]. The disease was not prevalent at Tule Lake in 1927, but there was an outbreak of moderate intensity in the fall of 1928 and localized outbreaks in each of the three following years.

In Oregon, duck sickness appeared in its most disastrous form at Lake Malheur in Harney County in 1925, a year of low water. It recurred in reduced intensity the following season; reached serious proportions again in 1929; and in 1930 and 1931 was absent, owing to the drying of the lake bed. The initial recorded outbreaks on bodies of water in parts of southern Oregon contiguous to the Tule Lake district of California appeared subsequent to 1925. These areas included marshes at the north end of Upper Klamath Lake, the Keno Marshes along Klamath River, and what is known as the Government sump, an irrigation catch-basin 10 miles south of Klamath Falls.

In Idaho the earliest recorded instance of duck sickness is that of an outbreak at Mud Lake, Jefferson County, in 1922, in the course

[10] TOMKIN, G. REPORT ON DUCK SICKNESS AT TULE LAKE, CALIF., KLAMATH LAKES, OREG., AND ADJACENT TERRITORY. 1926. (Manuscript in files of Bureau of Biological Survey.)

of which both wild and domestic ducks were affected. In 1929 mortality was again reported from the same general vicinity (Hamer). What appears to have been duck sickness has been noted at Bear Lake and the area just north of the Utah line (1926 and 1929) and at the American Falls Reservoir, Power County, where many birds died under essentially fresh water conditions in 1928. All these localities lie directly north of Great Salt Lake.

Lake Bowdoin, Phillips County, Mont., the scene of an outbreak reported by Wetmore (*71, p. 5–6*), again was the source of sick birds in 1921. George E. Mushbach, of the Biological Survey, encountered thousands of dead bodies there early in October of that year, evidence indicating a severe outbreak a little earlier in the season. What may have been duck sickness has been reported by Carey H. Bennett, of the Biological Survey, in recent years at Benton Lake, Cascade County; and Greenfield Lake, Teton County (not charted in fig. 1).

In Nevada there are two records to be considered in connection with the distribution of duck sickness. One of these emanates from the southeastern part of the State, where in the summer of 1930 several hundred birds died at Frenchmans Lake in the Pahranagat Valley, Lincoln County, under conditions that strongly suggested duck sickness. In the same season numerous avocets died on shallow alkaline lakes east of Fallon in the west-central part of the State. The evidence of duck sickness in this case, however, is not so clear.

Although the disease may have manifested itself at earlier dates in North Dakota, there is no question that it has been increasing in frequency during recent years. The center of the reported infection has been in the highly alkaline southern parts of Burleigh and Kidder Counties. In August 1929, F. M. Uhler, of the Biological Survey, noted two duck-sickness areas, one in Graf Township in southeastern Kidder County, and the other at Long Lake in the southwestern part of that county. At the latter point domestic chickens and turkeys also succumbed. In the following year large numbers of wild fowl died at Chase Lake Bird Refuge, in northern Kidder County, according to the report of Protector W. D. Parker. Earlier mortality at Cranberry Lake (1927) may not have been due to duck sickness but to the direct toxicity of alkaline salts, which were present in concentrations great enough to form dense crystaline deposits on the plumage of affected birds.

In South Dakota the history of duck sickness is recent, with the exception of the doubtful case occurring near Watertown in 1898 (p. 26). The first definite outbreak of which there is record was that reported by F. C. Lincoln, of the Biological Survey, at White Lake, Aurora County, in 1922. In 1927 the disease was reported from Rush Lake, near Webster, Day County, where it was learned the malady was of annual occurrence. Another Rush Lake, in Hamline County, was the scene of duck sickness in 1929, as was also Lake Poinsett nearby. No less than 20 species of birds, including a ring-necked pheasant, were found dead about this lake by Uhler in September, and he also encountered duck sickness at Lake Mitchell in Brookings County in the same year. From Antelope Lake, Clark County, there were received, in December 1930, specimens of mallards that evidently had died of lead poisoning, but the internal organs, after suitable incubation, yielded *Clostridium botulinum*, type C,

which indicated that the organism of duck sickness was present in that vicinity also.

In October 1931 Gunderson visited Oaks and Sylvan Lakes in Cottonwood County, Minn., and there found evidence of what appeared to have been an outbreak of typical duck sickness that had its peak at some time in August or September (*27*). Wild ducks, shore birds, and domestic poultry died in great numbers on the mud flats surrounding these lakes, and cultures made from material collected there revealed the presence of the organism. This is the most eastwardly record in the United States of duck sickness in epizootic form among wild birds in the region of alkaline water.

In Nebraska, duck sickness has been recorded at points in the sand hills of Garden and Sheridan Counties within this period, even as recently as 1932. In that year the disease was reported at Gay Lake, near Irwin, Cherry County; and at Whitney Lake, Dawes County.

In the Cheyenne Bottoms, Barton County, Kans., there appears to have been a similar malady in the fall of 1926. A much earlier report of mortality recorded by Wetmore (*71*, *p. 6*) as having occurred in this locality in 1914 and 1915 was at that time diagnosed at the University of Kansas as a bacterial infection.

In the Panhandle of Texas duck mortality came rather suddenly to the attention of sportsmen within recent years, although observers familiar with conditions contend that the malady has been an annual occurrence there over a longer period. It was reported as severe in the vicinity of Amarillo, Potter County, in 1926 and 1927, and prevalent at other points in western Texas during these years. In 1930 an intense but local outbreak appeared about small bodies of water near Cone, Crosby County. Desiccated specimens collected at these points in the year following afforded circumstantial evidence of the identity of the malady by the disclosure of the organism of type C botulism.

Waterfowl mortality in an overflowed area adjacent to the Rio Grande at San Acacia, N.Mex., (1927) resulted in the death of a few birds; as also at the Picacho Reservoir, Pinal County, Ariz., in October 1928. Although definite evidence is lacking, the circumstances suggest duck sickness.

A case of possible duck sickness was revealed at a point east of the Mississippi River when, on December 14, 1930, a single black duck was captured in a helpless condition near Dyke, Va., by W. H. Ball and H. S. Barber. Subsequent observation of the bird in captivity revealed a partial (probably residual) paralysis of some of the major muscles. After 2 days in captivity the bird was killed and its liver removed under sterile conditions. From this, Giltner recovered *Clostridium botulinum*, type C. Though there is the possibility that the liver contamination had its origin from bacteria adventitiously ingested, and was in no way connected with the bird's ailment, the symptoms were suggestive. The case serves at least as a definite locality record for the organism.

Mention may be made of the sickness and death of shore birds reported in September 1930 in Massachusetts Bay, where they were feeding in the vicinity of carcasses of blackfish (*Globicephala melaena*), which had been washed onto the beach and were undergoing

decay (*1*). Turnstones and sanderlings, feeding on the adult and possibly larval and pupal blowflies (*Calliphora*) infesting the carcasses, became afflicted with a paralysis of the major muscles and showed other symptoms of typical duck sickness. Although no bacterial evidence of the identity of this malady was obtained at the time, the circumstances surrounding the outbreak and the symptoms displayed point to botulism of some type. Incidents such as this, which are the direct result of a single local spot of infection, closely typify the conditions under which domestic poultry contract botulism and are not to be classed with the extensive epizootics among wild birds in the West.

Outside the borders of the United States, duck sickness has appeared in its most virulent and disastrous form in recent years in the southern portions of the Canadian Provinces of Saskatchewan and Alberta, directly north of Montana. Early in this period (1920 and 1921) a malady reported by Munro (*51, p. 83*) occurred among waterfowl at Lake Johnstone, Moose Jaw County, Saskatchewan, and was diagnosed at the time as coccidiosis and as septicemia. The symptoms, however, were similar to those exhibited by sick birds at Lake Newell (next mentioned) and in a present-day review of the evidence, duck sickness seems the logical diagnosis.

What appears to have been true duck sickness occurred for the first time at Lake Newell, Bow Valley County, Alberta, in 1924, and in the following year an epizootic of considerable severity ran its course in the same locality. The excellent and well-illustrated account of this outbreak by Munro leaves little doubt concerning the nature of the trouble (*51*). In 1926 no sickness was observed, and apparently it did not recur until 1930 when, according to A. Griffin, of Brooks, Alberta, a number of dead ducks were reported despite the fact that much fresh water had flowed into Lake Newell that season.

In Saskatchewan duck sickness was reported again (1927) at the point of its most northerly occurrence west of Saskatoon, and in 1928 there was some mortality at Lucky (Luck) Lake in Elrose County. In the following season, however, a malady in all its superficial aspects similar to duck sickness, made heavy inroads on the waterfowl of both southern Alberta and southern Saskatchewan. In that year many thousands died on the shores of Pakowki Lake, Medicine Hat County, Alberta, just north of the Montana border. At Many Island Lake, Redcliff County, Alberta, ducks died in July and August, it was stated, from the effect of leeches (p. 11), while at Cypress Lake, near Vidora, Saskatchewan, a severe malady occurred, the cause of which was uncertain.

South of the Rio Grande there is record of a malady that may have been duck sickness occurring in the fall of 1925 in northeastern Durango. There, according to Nelson (*52, p. 92*), in a district known as Bolson de Mapimi, which extends into adjacent southwestern Coahuila, formerly existed the Laguna de Meyran, a body of water more than 30 miles in length, and numerous smaller lakes. Diversion of water for irrigation has reduced the flow into this basin, which now is devoted largely to agriculture. The remaining marsh areas and flooded fields, however, are still a great attraction to waterfowl. In one section, near the town of Tlahualilo, many thousands of birds,

especially pintails, blue-winged teal, and sand-hill cranes, died in 1925 under conditions that indicated duck sickness. E. A. Goldman encountered no sickness when visiting this area in March 1926, but found considerable evidence of the previous year's mortality in the form of disintegrating bodies.

Early in the past decade (1921) Wetmore, while studying migratory birds in South America, found evidence of a malady among wild fowl in the vicinity of Laguna Castillos, near San Vicente de Castillos, Uruguay, the symptoms of which were the same as those of the disease he had studied in the Salt Lake Valley. His manuscript notes in the files of the Biological Survey, under date of January 31, 1921, show that several species were affected, including the southern pintail, a coot, Trudeau's tern, the brown-headed gull, and the lapwing, or teru teru (p. 41).

EXTENT OF DISTRIBUTION AND MORTALITY

The map (fig. 1) of the known distribution of duck sickness as an epizootic among wild birds in North America shows roughly a wide-flung circle having in its circumference points in the southern portions of the Canadian Provinces of Saskatchewan and Alberta, south-central Oregon, northern, central, and southern California, two areas in Nevada, possibly points in Arizona and New Mexico, and areas in the Panhandle of Texas, western Nebraska, eastern South Dakota, south-central North Dakota, and southwestern Minnesota. Near the center of this great circuit is Great Salt Lake, Utah, with infected areas of southern Idaho directly to the north. In the main this distribution, especially that part representative of the areas of greatest mortality, conforms roughly to the region of alkaline soils and waters of the United States. The significance of this correlation is discussed elsewhere (p. 63).

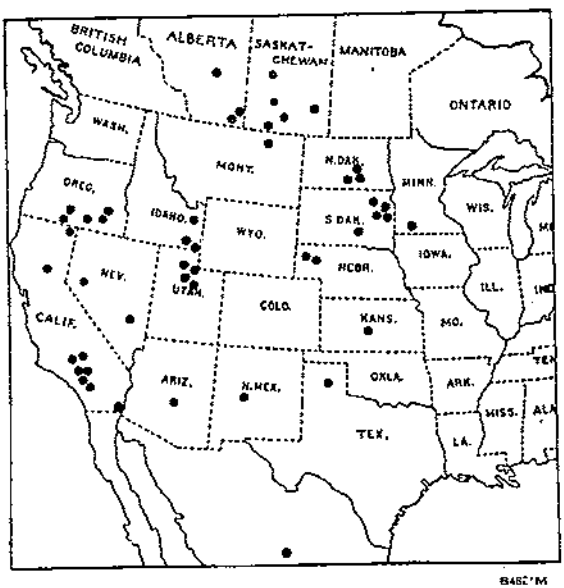


FIGURE 1.—Points from which duck sickness has been reported among wild birds. Bacteriological demonstration of botulism as the cause is limited to a very few of the localities, but the similarity of symptoms and significant environmental conditions have served as the basis of the records.

In the foregoing treatment by periods, duck sickness among wild birds has been traced with a fair degree of certainty as far back as the early nineties of the past century; its range now extends over an

enormous area. The history of events indicates an increase in prevalence; and from this comes the logical query as to whether a still further extension of range may be expected in the future.

That the causative organism of duck sickness, *Clostridium botulinum*, type C, is endemic in this continent and was here long before man recognized its presence through outbreaks of duck sickness, may be inferred from the studies of the same and other types of botulism investigated by bacteriologists. There is reason therefore to believe that duck sickness may have occurred at a very early time, whenever and wherever favorable etiological factors were present (pp. 61–68).

In looking backward, however, it is noted that man has altered greatly the extent and nature of conditions affecting duck sickness. Through reclamation, drainage, and deforestation there has resulted an increase in the rapidity and extent of seasonal run off of rainfall. Lakes and marsh lands that formerly maintained more or less constant water levels now fluctuate, and during late summer become stagnant areas of shallow water, mud flats, and decaying material. Alkaline sinks have been formed by drainage from irrigated sections. About many of these, grain crops have lured waterfowl in great numbers and have tended thereby to concentrate the birds in or near infected areas that formerly did not offer them much inducement to rest and feed. There is little doubt that there has been a material increase in the factors and general conditions conducive to duck sickness since civilized man has entered the picture; and, unless measures are taken to prevent a further extension of conditions favorable to the organism, the ravages of this disease are likely to make a still greater annual drain on our diminishing waterfowl.

Appraisals of the extent of mortality in severe epizootics were made as early as 1910 at Great Salt Lake, where sanitary conditions were being improved by gathering the dead bodies for burying or covering with lime. An idea of the intensity of the disaster may be had from the following description by Madsen (*45, p. 30–31*) of conditions about the lake in 1910:

> Our efforts were confined to gathering all the dead ones, loading them in flat-bottom boats with pitchforks, and hauling them to the nearest land to be piled up and buried or burned. . . . Three men with pitchforks accompanied each boat. I have seen many acres of water where these men could, within throwing distance, put in more than 200 birds without moving the boat. Small lakes would frequently yield more than 1,000 dead birds to the acre. We spent days at this work, until the utter uselessness of it all became apparent. The margins of the ponds and lakes soon became dotted with mounds of dead birds resembling rat dens in a marsh.

Wetmore has stated (*71, p. 3–4*) that in 1912—

> about 30,000 birds were picked up on the Weber River flats, while on Bear River, from records kept by V. F. Davis, it is learned that the bodies of 44,462 wild ducks were gathered and buried between August 22 and September 21.

Wetmore records in the following year that 46,723 ducks were buried between September 7 and September 26. He states further that "attempt was made to clean up only those birds lying in the open. These formed but a small part, so that the figures given probably represent less than 20 percent of the birds that actually died." Even in 1914, a year of considerable improvement over any of the preceding four, an estimate of 8,000 to 10,000 dead ducks was made

for a distance of 2 miles along one of the lower channels of the Weber River.

Others have attempted to appraise the extent of mortality by counting the number of dead and helpless birds on representative units and then computing the aggregate number on the basis of the total area affected. Such estimates may be subject to a great factor of error through inability to inspect much of the terrain, the tendency of bodies to be concentrated by wind and current action, and the habit of sick birds while still possessing powers of locomotion to hide in dense stands of marsh growth. Nelson (*53, p. 13*) states that on the Bear River Marshes, Madsen estimated the annual losses as from 30,000 to 100,000 or even more in years when the malady was particularly severe. There are seasons, however, when conditions of low water may result in the complete drying up of what are ordinarily duck-sickness areas, thus driving out the birds and preventing mortality. Such a condition prevailed in 1915, when Wetmore found sick birds scarce in some of the most severely infected areas of the Bear River Marshes. Estimates of dead ranging from less than 100,000 to more than 300,000 made at Great Salt Lake in August 1929, give a general idea of the severity of the epizootic in that year.

A rough estimate of the mortality at Lake Malheur in 1925, made by Steele, placed the number of dead at 100,000. At Tule Lake, Tonkin calculated the number of dead to be from 25,000 to 50,000 during the summer and fall of 1925. Early in the investigations of duck sickness in California an estimate of 40,000 dead waterfowl in one season (1913) was reported for the Buena Vista and Tulare Lake Basins (*8, p. 350*).

In the Klamath Falls–Tule Lake section of southern Oregon and northern California, nothing approaching the severity of outbreaks mentioned has appeared during the period of this investigation. In 1927 Sperry found no sick birds at Tule Lake, and only a few at other points in adjacent parts of southern Oregon. In the following year, 1928, an outbreak of moderate intensity ran its course late in the season. In 1929, 1930, and 1931 the mortality in the area was confined largely to overflowed land adjacent to Tule Lake, where it was estimated that about 1,000 birds died annually.

At the close of the severe, late-season outbreak at the north end of Great Salt Lake in 1932, an estimate was made of the number of dead on the south shore of Willard Spur. Sections of the shore line 100 yards in length were taken as units and the readily visible dead counted. For a distance of 6 to 8 miles dead ducks were scattered on the flat terrain in numbers ranging from 8,000 to 10,000 to the mile. (See pl. 1, *B*.) It was estimated that there were 150,000 dead on the south shore of Willard Spur and the adjacent Bear River Bay. Many more thousands lay scattered on the east, north, and west shores, and an indeterminate number were hidden in the vegetation of the Bear River Migratory Bird Refuge or obliterated on the mud flats. It was estimated that fully a quarter of a million waterfowl perished from duck sickness in this general area during 1932.

A limited to moderate mortality probably can be found in most of the principal infected areas every season. Epizootics of extreme

severity may be looked upon as the result of the combination of various etiological factors coupled with the attendant factor of an abundance of birds (*63*).

SPECIES OF BIRDS AFFECTED

At the conclusion of his work in Utah, Wetmore listed 36 species of wild birds known to be susceptible to duck sickness. With all but one of these, the horned lark, he had had personal contact in the course of his studies on the Bear River marshes and elsewhere. It was Wetmore's experience (*71, pp. 11–12*) that the shallow-water, or river, ducks were more likely to contract the disease than others. The green-winged teal was considered to be particularly susceptible, followed closely by the pintail; while mallards, shovelers, and cinnamon teal succumbed less frequently. Of the deep-water ducks, the redhead was most often afflicted; and among shore birds, the avocets and black-necked stilts died in greater numbers.

In a tabulation of each species found dead at Tulare Lake in 1918, published by Clarke (*8, p. 220*), of 2,079 recorded, 642 were teals (presumably cinnamon and green-winged), 571 were pintails, and 540 shovelers. Sandpipers of several species aggregated 46, and other birds were found in smaller numbers. No mallards were recorded, a circumstance that can be interpreted only by the fact that few if any were in the vicinity of the lake at the time.

The record of mortality in southern Oregon and northern California in the period 1929-31 parallels in the main that noted by Clarke at Tulare Lake more than 15 years earlier. In point of numbers, pintails were the ducks most frequently affected, although a variation was noted between one season and another and at different periods during the same season. In 1931 there was a noticeable increase in the number of dead green-winged teal about August 20. Within a week, pintails again took the lead and died in greater numbers than had previously been noted in the same area. Sick shovelers frequently were encountered there and, by the low percentage of recovery revealed apparently a weak resistance.

The relative status of mallards, cinnamon teal, and redheads with respect to the disease about Tule Lake was closely similar to that described by Wetmore for these birds at Great Salt Lake; with shore birds, however, a marked difference was noted. Although in 1929 and 1930 avocets dominated the number succumbing at Tule Lake, in 1931 least and western sandpipers clearly took the lead. Mortality among gulls never was extensive at this lake, but at an irrigation basin south of Klamath Falls, juvenile ring-billed and California gulls supplied the first evidence of duck sickness in each of the three seasons devoted to the study. After this early-season mortality, gulls afflicted with duck sickness were found rather infrequently.

The accompanying annotated list of wild birds known to have contracted duck sickness under field conditions gives a more detailed picture of the mortality aspects of the outbreaks. Bacteriological proof of the identity of the malady that killed the birds of each species noted is, in most cases, lacking, the criterion for inclusion

being similarity of symptoms and certain associated etiological factors characteristic of outbreaks of true duck sickness. The species marked with an asterisk (*) were included in Wetmore's original list (*71, p. 12*). At present the number of species of North American wild birds known to have been affected by duck sickness totals 69, in 21 families.

*EARED GREBE. *Colymbus nigricollis californicus.*

A few dead were noted at Lake Malheur, Oreg., in September 1929.

*WESTERN GREBE. *Aechmophorus occidentalis.*

Found affected at Long Lake in southwestern Kidder County, N.Dak., by Uhler, in August 1929; at Lake Newell, Alberta, by Munro, in 1925; and at Lake Malheur, Oreg., by Kalmbach, in September 1929. Extensive mortality reported among these birds at Owens Lake, Calif., at an early date (1891) may not have been due to duck sickness (p. 25).

*WHITE PELICAN. *Pelecanus erythrorhynchos.*

FARALLON CORMORANT. *Phalacrocorax auritus albociliatus.*

Included on the basis of affected individuals recorded by Clarke, at Tulare Lake, Calif., in 1913 (*9, p. 220*). Occurrence of this outbreak at about the time the last of the fish in the lake perished, apparently from stagnant water conditions, may account for birds of this highly piscivorous species contracting the malady.

TREGANZA'S HERON. *Ardea herodias treganzai.*

Specimens of sick herons were encountered at Great Salt Lake by Lincoln in 1926, and in the Klamath Falls district by Sperry in 1927.

*SNOWY EGRET. *Egretta thula thula.*

BLACK-CROWNED NIGHT HERON. *Nycticorax nycticorax hoactli.*

Night herons, dying apparently from duck sickness, were observed at Long Lake, Kidder County, N.Dak., by Uhler in August 1929, and about Klamath Falls by Sperry in 1927.

*WHITE-FACED GLOSSY IBIS. *Plegadis guarauna.*

*CANADA GOOSE. *Branta canadensis canadensis.*

Reported affected also at Lake Newell, Alberta, by Munro in 1925 (*51*). A few succumbed about Tule Lake, Siskiyou County, Calif., in the course of this investigation.

WHITE-FRONTED GOOSE. *Anser albifrons albifrons.*

Birds of this species occasionally succumb to duck sickness about Tule Lake, but ordinarily they arrive from the north after the seasonal peak of the disease. Sick "speckle-bellies" were found there in 1926, 1927, and 1930.

*COMMON MALLARD. *Anas platyrhynchos platyrhynchos.*

Sick mallards have been found in all principal duck-sickness areas and from the earliest known outbreaks to the present.

COMMON BLACK DUCK. *Anas rubripes* subsp.

Included on the basis of a single specimen collected on the Potomac River below Washington, D.C., December 14, 1930. This bird manifested certain residual paralytic symptoms occasionally found in duck sickness and later *Clostridium botulinum*, type C, was isolated from its internal organs (p. 30).

*GADWALL. *Chaulelasmus streperus.*

Recorded as succumbing, but never in great numbers, at Great Salt Lake, Utah; in North Dakota; at Lake Newell, Alberta; and in the Klamath Lake district in southern Oregon.

*BALDPATE. *Mareca americana.*

Found dead in most of the principal duck-sickness areas. At Tule Lake, Calif., the number afflicted, though limited, was large in proportion to the total number of this species present.

*AMERICAN PINTAIL. *Dafila acuta tzitzihoa.*

Probably more pintails succumb to duck sickness than any other single species. Locally or periodically other birds may contribute more to the general mortality, but the breeding range and late-summer concentration and migration movements throw this bird in frequent contact with disease areas. This is particularly true in central and southern Oregon and throughout California.

*GREEN-WINGED TEAL. *Nettion carolinense.*

Green-winged teal die in great numbers at all principal points of infection. When birds are being rescued the little teal often succumb in the process of handling. One seldom finds utterly prostrate teal in the field—they usually are mildly affected, possibly just contracting the disease, or are dead.

BLUE-WINGED TEAL. *Querquedula discors.*

Dead birds reported from Kidder County, N.Dak., by Uhler in August 1929; and from Lake Newell, Alberta, by Munro in 1925.

*CINNAMON TEAL. *Querquedula cyanoptera.*

A common bird about Tule Lake, Calif., through the seasonal period of sickness. Fewer cinnamons, however, perish than green-wings, which flock in during migration.

*SHOVELER. *Spatula clypeata.*

Sick "spoonbills" were reported from all the main duck-sickness areas. They display a relatively low resistance to the disease, and many perish even though rescued and given good care.

*REDHEAD. *Nyroca americana.*

Observations at Tule Lake bear out those by Wetmore at Great Salt Lake to the effect that redheads, particularly birds of the year, perish in large numbers. It would appear that the feeding habits of these young lead them to ingest toxic material more frequently than do adults.

RING-NECKED DUCK. *Nyroca collaris.*

Archie V. Hull has found a few ring-necked ducks afflicted with duck sickness on the Bear River Marshes, Utah, in recent years.

CANVASBACK. *Nyroca valisineria.*

In the severe late-season outbreak of 1932 in Willard Spur, Utah, numbers succumbed to the disease. These birds do not ordinarily arrive in the infected areas about Great Salt Lake until after the malady has subsided.

LESSER SCAUP DUCK. *Nyroca affinis.*

The migration of "bluebills" is not early enough to bring many into sickness areas in this country until after the most toxic period. Sick birds have been reported, however, at Lake Newell, Alberta, by Munro in 1925; and at the Bear River Migratory Bird Refuge, Utah, by Hull in more recent years.

AMERICAN GOLDEN-EYE. *Glaucionetta clangula americana.*

The golden-eye has been found a victim of duck sickness at Lake Malheur (September 1929) and at Great Salt Lake (November 1932).

BUFFLE-HEAD. *Charitonetta albeola.*

In November 1932, a number of these birds were afflicted with duck sickness in areas adjacent to Willard Spur, at the north end of Great Salt Lake. Usually the disease has disappeared by the time these late migrants arrive in numbers.

*RUDDY DUCK. *Erismatura jamaicensis rubida.*

Not a common victim of duck sickness.

RED-BREASTED MERGANSER. *Mergus serrator.*

In November 1932, one dead and several affected mergansers which, in all probability, had ingested toxin were found in the Willard Spur section of Great Salt Lake.

MARSH HAWK. *Circus hudsonius.*

Marsh hawks affected apparently by botulism have been noted by Lincoln, at Big Alkali Lakes southeast of Dawson, N.Dak. (1929); by Hull, on the Bear River Marshes, Utah; and by Kalmbach, at the mouth of the Blitzen River,

Lake Malheur, Oreg. (1929). The carrion-feeding habits occasionally indulged in by these birds may explain the contraction of the malady. One partially paralyzed and flightless individual was found at Lake Malheur stranded on a small clump of tules to which the current had drifted the body of a teal on which it was feeding.

PRAIRIE FALCON. *Falco mexicanus.*

A juvenile individual found dead on the algal beds of Tule Lake, October 10, 1930, is the basis for including this species among the victims of duck sickness. The bird was in excellent flesh, with no signs of death by shooting or bodily injury, nor with any marked pathological lesions, a characteristic condition of birds succumbing to botulism. A culture made of its liver, removed under sterile conditions, revealed *C. botulinum*, type C. The fact that the bird was young would account for the possibility of this naturally predacious species having fed on a dead specimen in which toxin had been formed.

DUCK HAWK. *Falco peregrinus anatum.*

One bird found by Hull on the Bear River Marshes, was in a helpless condition and exhibited all the characteristic symptoms of duck sickness.

RING-NECKED PHEASANT. *Phasianus colchicus torquatus.*

Included on the basis of observations made by Uhler at the scene of an outbreak of duck sickness at Lake Poinsett, Hamlin County, S.Dak., in September 1929.

*AMERICAN COOT. *Fulica americana americana.*

A frequent victim of duck sickness, yet in the outbreaks encountered by Kalmbach the number succumbing was proportionately small compared with the total number of coots present.

*KILLDEER. *Oxyechus vociferus vociferus.*

An occasional victim at Great Salt Lake and in the areas covered by this study, but at Lake Newell, Alberta, killdeers comprised about 4 percent of the affected birds in 1925 (*51, p.* 78).

AMERICAN GOLDEN PLOVER. *Pluvialis dominica dominica.*

A single specimen, apparently affected with duck sickness, was picked up on the Bear River Marshes by Hull in the summer of 1932.

BLACK-BELLIED PLOVER. *Squatarola squatarola.*

Affected birds have been reported within recent years by Munro at Lake Newell, Alberta (*51, p.* 78), and by Hull at Great Salt Lake, Utah.

RUDDY TURNSTONE. *Arenaria interpres morinella.*

Reported affected at Lake Newell, Alberta (*51, p.* 78).

EASTERN SOLITARY SANDPIPER. *Tringa solitaria solitaria.*

Noted sick by Uhler, at Long Lake, Kidder County, N.Dak., in August 1929.

WESTERN WILLET. *Catoptrophorus semipalmatus inornatus.*

Reported affected at Lake Newell, Alberta, by Munro, in 1927, (*51, p.* 78); and at Long Lake, southwestern Kidder County, N.Dak., by Uhler, in 1929.

*LESSER YELLOW-LEGS. *Totanus flavipes.*

A few have been found affected in several of the important sickness areas.

AMERICAN KNOT. *Calidris canutus rufus.*

The single record is based on a specimen found by Munro, at Lake Newell, Alberta, in 1925.

*PECTORAL SANDPIPER. *Pisobia melanotos.*

BAIRD'S SANDPIPER. *Pisobia bairdi.*

Reported affected and dying in considerable numbers at Lake Newell, Alberta (*51, p.* 78).

*LEAST SANDPIPER. *Pisobia minutilla.*

Recorded dying in limited numbers at Great Salt Lake by Wetmore. A few dead specimens also were collected by Uhler at Long Lake, Kidder County, N.Dak., in 1929. Extensive mortality occurred at Tule Lake, Calif., in the sum-

mer of 1931 among these diminutive shore birds which were obtaining much of their food from the larvae and pupae of hydrophilid beetles, the cocoons of which were formed on the upper surface of the algal beds (p. 13). A considerable number also were found dead on the borders of overflowed wheatlands northwest of Tule Lake in the summer of 1929.

*RED-BACKED SANDPIPER. *Pelidna alpina sakhalina.*

Recorded by Wetmore as dying at Tulare Lake, Calif.

*LONG-BILLED DOWITCHER. *Limnodromus griseus scolopaceus.*

Not a frequent victim of duck sickness.

STILT SANDPIPER. *Micropalama himantopus.*

Recorded by Munro as dying at Lake Newell, Alberta, in 1925.

*WESTERN SANDPIPER. *Ereunetes mauri.*

These birds possessed the same habits and shared the same fate as the least sandpipers at Tule Lake, Calif., in 1931.

*MARBLED GODWIT. *Limosa fedoa.*

At Lake Newell, Alberta, duck sickness has been recorded as occasionally destructive to marbled godwits. At that lake in 1925 these birds comprised more than 6 percent of the total dead (*51, p. 78*).

SANDERLING. *Crocethia alba.*

A single dead specimen was recorded by Munro in the course of the outbreak at Lake Newell, Alberta, in 1925. In recent years the species also has been found affected at Great Salt Lake by Hull.

*AVOCET. *Recurvirostra americana.*

Probably more avocets die of duck sickness than any other species of shore bird. This statement is made in full cognizance of the fact that sick or dead avocets are conspicuous objects and are easily detected while the least and western sandpipers which also die in great numbers may easily pass unnoticed. Affected avocets have been reported from all the principal duck-sickness areas. They early lose control of their leg muscles and, once down, seldom regain the ability to stand, even under conditions favorable to their recovery.

* BLACK-NECKED STILT. *Himantopus mexicanus.*

Occasional sick or dead stilts were found in the course of the field work incident to this study. Wetmore, however, found them dying about Great Salt Lake in numbers second only to the avocet among the group of shore birds. Once they have lost the ability to stand, they are doomed, since, aside from the effect of the disease itself, the birds often are confronted with feather soaking, and entanglement in the masses of algae, from which they seldom free themselves.

WILSON'S PHALAROPE. *Steganopus tricolor.*

Sick birds have been found at Tule Lake, Calif., by Kalmbach; and at Great Salt Lake, Utah, by Lincoln, in 1926.

NORTHERN PHALAROPE. *Lobipes lobatus.*

In 1929 a considerable number of migrating individuals came to Tule Lake at a time when duck sickness still was prevalent, and a few contracted the disease.

PARASITIC JAEGER. *Stercorarius parasiticus.*

A single individual affected apparently with duck sickness, was found in 1932, by Hull on the Bear River Marshes, Utah.

* CALIFORNIA GULL. *Larus californicus.*

The first indication of duck sickness in each of the three seasons devoted to the study was manifested among juvenile gulls on an island in an irrigation catch basin, 10 miles south of Klamath Falls, Oreg. In 1930 and 1931 young California gulls were the first to succumb, and in 1929 a young ringbill gave first indication of the seasonal outbreak. These young gulls were raised on a mixed diet of insects (mainly grasshoppers), crawfish, and fish. The nesting ground was littered with uneaten or regurgitated food, which easily might serve as a suitable incubating medium for the toxin of botulism. The greater part of this early mortality was confined to the young gulls; the older birds

apparently were not ingesting toxic material so frequently. Later in the season when gulls were found generally distributed throughout the irrigated sections and about Tule Lake, only an occasional sick one was found. This gull has also been reported as succumbing at Great Salt Lake, Utah (*71*), and at Lake Newell, Alberta (*51*). The fact that more of these carrion feeders are not killed may be due to their tolerance of the toxin (p. 44).

* RING-BILLED GULL. *Larus delawarensis.*

Observations with respect to ring-billed gulls and duck sickness closely parallel those made on the California gull.

FRANKLIN'S GULL. *Larus pipixcan.*

Munro found Franklin's gulls dying in considerable numbers in the outbreak of duck sickness at Lake Newell, Alberta, in 1925 (*51*), and Uhler and Lincoln reported them at two points in Kidder County, N.Dak., in 1929. A single bird found sick at Tule Lake, Calif., July 31, 1931, displayed all the characteristic symptoms of the disease, and when well on the road to recovery suffered a relapse, precipitated perhaps by its having been fed the flesh of other birds that had died of the disease.

BONAPARTE'S GULL. *Larus philadelphia.*

The only known cases of duck sickness in this species were two sick and several dead individuals found at Tule Lake, Calif., July 31, 1931.

*FORSTER'S TERN. *Sterna forsteri.*

*BLACK TERN. *Chlidonias nigra surinamensis.*

Individuals dying apparently from duck sickness have been found at Great Salt Lake (Wetmore) (*71*); at Long Lake, Kidder County, N.Dak. (Uhler); and at Lake Newell, Alberta (Munro) (*51*).

*HORNED LARK. *Otocoris alpestris* subsp.

Found affected at Lake Bowdoin, Mont. (*71*).

*NORTHERN CLIFF SWALLOW. *Petrochelidon albifrons albifrons.*

On the Bear River Marshes, Utah, Wetmore found several swallows that revealed typical symptoms of duck sickness. D. D. McLean also has reported sick cliff swallows in California (as published by Hobmaier (*40, p. 11*)).

*AMERICAN MAGPIE. *Pica pica hudsonia.*

This is one habitual carrion feeder that has not developed an immunity to type C botulism. Wetmore noted sick magpies on the Bear River Marshes in Utah, that frequently had fed on bodies of birds that had died of duck sickness.

*AMERICAN PIPIT. *Anthus spinoletta rubescens.*

WESTERN MEADOWLARK. *Sturnella neglecta.*

Reported affected in California by McLean (*40, p. 11*).

* YELLOW-HEADED BLACKBIRD. *Xanthocephalus xanthocephalus.*

In addition to Wetmore's record, Sperry found afflicted yellowheads in the Klamath Falls area in 1927.

NEVADA RED-WINGED BLACKBIRD. *Agelaius phoeniceus nevadensis.*

Redwings afflicted apparently with duck sickness were noted by Sperry in the Klamath Falls area in 1927.

* RUSTY BLACKBIRD. *Euphagus carolinus.*

Reported by Wetmore at Lake Bowdoin, Mont.

BREWER'S BLACKBIRD. *Euphagus cyanocephalus.*

Afflicted individuals were found in the Klamath Falls area in 1927, 1929, and 1931, on ditch banks adjacent to overflowed land where ducks were dying in considerable numbers.[11]

[11] In addition to this list, mention may be made of two other species recorded in the literature of botulism: Dickson (*15*) has noted the susceptibility of a "wild canary" (goldfinch, *Spinus* sp.); and Hart (*33*) that of the California linnet, or common house finch (*Carpodacus mexicanus frontalis*). In each of these instances, however, the virulent toxin of type A botulism may have been involved.

Besides the foregoing records of species of North American wild birds afflicted with duck sickness, mention should be made of several South American birds known to have succumbed to the same malady under field conditions. These records were submitted by Wetmore, who from his earlier studies at Great Salt Lake was thoroughly familiar with the symptoms of the disease. He encountered an outbreak of duck sickness while engaged in ornithological studies in Uruguay in 1921. At Laguna Castillos, near San Vicente de Castillos, about 400 miles east-northeast of Buenos Aires, he recorded on January 31, 1921, the following:

> The shores of the lake were alkaline. . . . I noticed a few dead coots strewn along without paying particular attention until I picked up a living brown-headed gull (*Larus maculipennis*) in a helpless condition. Struck by its appearance, I examined it closely and was astonished to discover the unmistakable symptoms of duck sickness. . . . A short distance farther on I found a Trudeau's tern (*Sterna trudeaui*) in the same condition. About 40 coots (*Fulica armillata*), 10 southern pintail (*Dafila spinicauda*), 2 lapwing (*Belonopterus chilensis lampronotus*), and 2 brown-headed gulls were found dead in a distance of a quarter of a mile.

Domestic poultry may contract duck sickness in environments and in a manner similar to that experienced by wild birds. It would appear that domestic ducks of all breeds may succumb when they have access to areas where toxin is being produced. Late in the summer of 1928 an entire flock of domestic ducks of several breeds, belonging to a rancher living southwest of Klamath Falls, Oreg., perished from what appeared to have been typical duck sickness. These birds had had access to an alkaline pond about which shore birds also died. In 1931 the body of an avocet found on a small island in this pond revealed *Clostridium botulinum*, type C.

During a severe outbreak of duck sickness at Long Lake, southwestern Kidder County, N.Dak., in 1929, Uhler noted mortality among domestic chickens and turkeys that had wandered into an area where wild birds were affected. Similar mortality was reported near Cone, Crosby County, Tex., during an outbreak in 1930. Instances such as these indicate that conditions that produce duck sickness among wild birds likewise may constitute a direct menace to domestic birds. Under the conditions of domestication the contraction of duck sickness (limberneck) ordinarily is associated with the presence of localized sources of toxin, as individual carcasses of birds or mammals to which poultry have access. When these are removed the trouble ceases. Domestic ducks have been known to perish under conditions of general stagnation created in small artificial ponds in which the essential features of a naturally infected environment are closely simulated. Mention may be made of the death of a number of Nile geese, gray-backed ducks, and other species in the National Zoological Gardens, in Pretoria, South Africa, in December 1928 (*56*). A similar outbreak had taken place 3 years before but was not investigated. Although the actual toxin-producing medium was undetermined, the suggestion was made that the bodies of earthworms may have supplied it. Cultures made from material collected in this outbreak had not been typed at the time the initial report on the case appeared, but it has been learned, through subsequent correspondence with E. M. Robinson, of the Department of Agriculture

of the Union of South Africa, that all avian cases of botulism investigated in South Africa have been of type C.

A somewhat similar case, resulting in the death of numerous ducks and two swans, has been reported in American literature (*54*). In this instance reported by Palmer and Baker, in 1922:

> The lake was stocked with fish. About the middle of September numerous dead fish and tadpoles were observed floating along the shores. Some of the dead tadpoles and small fish were ingested by the ducks and swans, and soon thereafter the ducks began showing symptoms of botulism . . . One swan died in about 24 hours after showing clinical symptoms of botulism.

The type of botulism involved in this outbreak was not determined.

Botulism naturally contracted has been recorded in domestic ducks of unnamed variety and ostriches in South Africa (*66, p. 1292*). It was inferred that the ducks had ingested carrion and that the ostriches had contracted the malady by feeding on bones in which toxin had been produced.

Several species of birds have been employed in experimental work with type C botulism. Chickens of various breeds, as well as domestic ducks, have been used by research workers in the study of limberneck in this country. Domestic ducks, turkeys, and pigeons, as well as ostriches, have served the same purpose with respect to lamsiekte in South Africa. In the course of the present study domestic pigeons were used almost exclusively in the course of bacteriological work. It was found that they could be easily obtained and kept in captivity; that they are readily susceptible to doses administered interperitoneally; and, through their use, the employment of valuable migratory waterfowl in experimental work was obviated.

SUSCEPTIBILITY OF BIRDS

Little is known of the actual or relative susceptibility of different species of wild birds to type C *botulinum* toxin. Extent of mortality or affliction as encountered in the field cannot be taken as an index, for the reason that numerous factors other than abstract susceptibility play important roles in its determination. The number of affected birds that will be found is usually in proportion to the abundance of each species on the infected areas. The sudden influx of a great number of migrants, with a resultant increase in mortality, has led some observers to conclude that nonresident birds are more susceptible than those reared in infected areas. The present investigation has brought forth nothing, however, that would indicate immunity in birds after one or more contacts with the disease in the field.

Food preferences or feeding habits also may throw this or that species into more or less intimate contact with the toxin of botulism. Late in the summer of 1930, and again in 1931, thousands of Canada and white-fronted geese frequented areas adjacent to Tule Lake, where ducks and shore birds were dying in considerable numbers, yet in the course of the two seasons only five sick geese were found. The browsing and grainfield-feeding habits of these birds in distinction from the puddling habits of the ducks quite effectively prevented their ingesting the toxin generated on the infected mud flats. During the season of 1931 the many least and western sandpipers that

perished at Tule Lake were feeding extensively on larvae and pupae of hydrophilid beetles in cocoons formed in the masses of algae on which these diminutive shore birds walked in search of food. The finding of the remains of many of these larvae in the stomachs of the dead, coupled with the demonstration of *Clostridium botulinum*, type C, in some of the dead larvae, strongly suggests the particular medium through which these birds, and probably few other species, obtained a lethal dose of toxin. At the same time and in close proximity, large flocks of long-billed dowitchers, probing in the mud beneath a shallow but moving sheet of water, almost completely escaped the malady, either because they were not regularly ingesting suitable toxin-producing food or because the steady flow of water was diluting preformed toxin to a degree that made it harmless.

That the extent of mortality cannot be used as an index to the degree of susceptibility is further brought out by the fact that, in judging abstract susceptibility the element of body weight must be considered. Although it is apparent that larger birds would ingest relatively greater quantities of toxic material in feeding it is obvious that under the varied vicissitudes confronting the birds in the field, the ratio would be by no means constant. For instance, to demonstrate relative susceptibility between least sandpipers and Canada geese on the basis of the numbers of dead found, one would have to show, among all other factors previously mentioned, that the geese were regularly ingesting about 150 to 200 times as much toxic material as the sandpipers.

An insight into the matter of relative and actual susceptibility was obtained, however, in the course of experimental work with captive birds. To determine relative susceptibility, an experiment was conducted with 8 green-winged teal and 16 pintails, injected intraperitoneally with a diluted, filtered culture of type C *botulinum*. It was found that the minimum lethal dose of this rather weak filtrate was about 0.025 cc for the green-winged teal (average weight, 255 g), and 0.05 cc for the pintails (average weight, 600 g). This indicated little difference, when body weight is taken into consideration, in the susceptibility of the two species.

An index of the relative susceptibility of these ducks and of domestic pigeons was gained from an experiment run concurrently with the one just described and with the same lot of toxin, so there could have been no differences from changes in potency of culture. Sixteen pigeons were employed, each weighing approximately 350 g. It appeared that the minimum lethal dose for pigeons was not far from 0.0002 cc of this filtrate injected intraperitoneally. From the experiment previously discussed, it may be inferred that the minimum lethal dose for the green-winged teal, weighing about 255 g, is approximately 125 times that for pigeons. Likewise it may be concluded that the minimum lethal dose for the pintail is about 250 times that for pigeons. Converting these manifestly meager data into an appraisal of abstract susceptibility to intraperitoneal doses, by allowing for the element of body weight, it may be concluded that in general the domestic pigeon (350 g) is 175 times as susceptible to the toxin of type C botulism as the green-winged teal (255 g), and 146 times as susceptible as the pintail (600 g).

In the course of experimental work in 1930 an insight was obtained regarding the relative susceptibility of gulls and ducks to oral doses. Extensive use was then made of the incubated livers of birds that had died of duck sickness. This served as an excellent toxin-producing medium, and by feeding it in capsules to experimental birds it was found that botulism could be produced (pp. 55–56). In one experiment 0.5 g of such toxic liver, of which an equivalent amount fed orally had killed a mallard, failed to produce symptoms in a juvenile ring-billed gull. In other experiments it was demonstrated that juvenile ring-billed gulls are at least 20 times more tolerant to oral doses of type C toxin than are adult pintails.

This marked difference in susceptibility of ducks and gulls to the oral assimilation of the toxin is not duplicated when the toxin is administered by injection. Experiments in which ring-billed gulls, mallards, and pintails were used indicated that the minimum lethal dose by injection varied little one from another.

An idea of the quantity of toxic material of moderate potency that must be ingested by wild birds to produce the disease was gained from feeding experiments, in which the incubated livers of birds dying of the disease were used. It was found that 0.1 g of such material given orally to a pintail produced a typical, protracted, and fatal case of duck sickness. In another instance 0.2 g of toxic liver produced a typical sublethal case in a female domestic mallard weighing 1,162 g, but the same dose did not affect a 1,717-g male domestic mallard. In 8 experiments 0.25 g of such toxic liver was sufficient to kill pintails and mallards, and in 4 others less toxic material, in equivalent doses, produced typical symptoms but not death. In no case in which the material was demonstrated to be toxic did a duck survive an oral dose as great as 0.5 g. As this quantity of material bulks about the size of an average pea, it will be seen that the lethal dose for a duck feeding in the field on any equally toxic material is relatively small. Such results lead to the conclusion that in the field, where a high percentage of the naturally produced cases are sublethal, the birds must be ingesting either very small quantities, or that the toxin involved is of low potency. Hobmaier has indicated the possibility of producing the disease by repeated small doses, any one of which would have no effect (*40*).

As few as 10 small dead sarcophagid larvae, weighing together only 0.1 g, contained enough toxin to kill a male pintail 4 days after they were ingested. Fifteen somewhat larger sarcophagid larvae fed to each of two mallards caused death in 19 and 36 hours, respectively. When in later bacteriological work a standardized beef-heart-peptone medium was used, 0.5 cc administered orally was found to kill a pintail in about 45 hours.

Gunnison and Coleman (*29*) demonstrated that the minimum lethal dose for pintails was from 100,000 to 200,000 guinea-pig minimum lethal doses of toxic culture, given by mouth. They also showed that the ratio of the injected to the oral minimum lethal dose of this strain of type C *botulinum* is, for the pintail, from 1:10 to 1:50. This is in contrast to a proportionately much greater oral dose recorded for small laboratory mammals (p. 47).

Pigeons studied in relation to lamsiekte in South Africa, proved decidedly refractory to the toxin of this cattle disease both when treated orally and subcutaneously (*66, p. 1115*). It is possible that future studies may corroborate this marked difference in the action of type C of Bengtson and of type D, which is now looked upon as the bovine strain in South Africa.

The marked resistance of chickens to the toxin of type C botulism administered orally has been demonstrated. Graham has shown that some chickens may without ill effect ingest in single massive doses as much as 1 g of toxic culture to 30 g of body weight (*26, p. 28*). Bengtson (*3, pp. 68–69*) reports that 250,000 guinea-pig minimum lethal doses fed to a 1,180-g chicken produced severe symptoms, though the bird recovered, and comments further on type C toxin to the effect that "the toxin in carrion material or fly larvae is probably in much more concentrated form." In any event, the effects produced on chickens feeding under natural conditions, as in the case of wild birds, are dependent on the quantity of material eaten. Bengtson records that 25 cc of a culture in a cooked-meat medium, fed to a 1,200-g chicken, produced typical symptoms in 70 hours and eventually death. The resistance of chickens has been noted even when the toxin of type C is administered by injection, although the minimum lethal dose in such cases is somewhat less than a tenth of the lethal oral dose.

Of interest in the matter of avian susceptibility to botulism is the reaction of the turkey buzzard, which, by reason of its feeding habits, has for ages been thrown in intimate contact with the toxin elaborated by *Clostridium*. Through the cooperation of officials of the National Zoological Park, Washington, D.C., four of these birds were obtained for experimentation. An oral dose of 30 cc of a type C culture (of which a 0.0001-cc intraperitoneal dose was sufficient to kill a pigeon) failed to have any effect. Intraperitoneal doses of 1, 5, and 10 cc likewise produced no symptoms, and a dose of 15 cc caused only a slight, temporary weakness in the legs and wings about 24 hours after injection. A culture of type A of fair potency (the minimum lethal dose for a guinea pig being between 0.001 and 0.0001 cc, intraperitoneally) also produced no effects with injected doses of 1 and 5 cc. However, 10 cc caused a slight, temporary impairment of locomotion 2 days after administration. The most startling results were obtained with a highly potent type B culture (the guinea-pig minimum lethal dose being 0.00005 cc by injection). Intraperitoneal doses of 5, 10, and 15 cc failed to produce any noticeable symptoms. The last of these doses was the equivalent of 300,000 guinea-pig minimum lethal doses, and indicated a tolerance expressable by the ratio of 0.04 cc of injected toxin for every gram of body weight.

OTHER ANIMALS AFFECTED

Little is known of the effect of duck sickness under field conditions on wild creatures other than birds. Wetmore (*71, p. 12*) stated that duck sickness affected muskrats and that at times frogs (*Rana pipiens*) died apparently from the same cause at Great Salt Lake.

He also suggests that even certain aquatic beetles (*Cybister* and *Dytiscus*), predacious in habits, may have been affected.

Dead muskrats were found in 1929 at the scene of a duck-sickness outbreak in Day County, S.Dak., by Uhler. In the same year W. H. Ransom reported muskrats dying near Hamer, Idaho, in the course of a similar outbreak. In the summer of 1927 Sperry found a dead weasel (*Mustela*) near Klamath Falls under conditions indicating that it might have contracted duck sickness through feeding on the bodies of dead ducks. In none of these instances involving the death of vertebrates, other than birds, were symptoms noted, nor was bacteriological evidence of the nature of the malady obtained. It is important, however, to consider the possibility of mortality among lower and more abundant forms of life, not only because of its direct effect on these, but also because, by the death of large numbers of small inconspicuous forms, the quantity of possible toxin-producing media is increased.

It has been well established that the more toxic forms of A and B *botulinum* affect a great variety of organisms, ranging from copepods, earthworms, snails, tadpoles, frogs, and fishes (*57*) to higher vertebrates, including such diverse forms as rats, cats, dogs, pigs, horses, goats, cattle, monkeys, and man himself. Less is known of the susceptibility of organisms to the toxin of type C, yet experimental work has brought out the fact that numerous vertebrates other than birds, as well as possibly certain invertebrates, are susceptible to the toxin of the type causing duck sickness. In the course of the present investigation evidence pointed to the susceptibility of copepods (*Cyclops serrulatus*). Inconclusive results were obtained in experiments with snails (*Goniobasis silicula*) and crawfishes (*Astacus klamathensis*). Toads (*Bufo boreas*) were refractory to oral doses, and even when toxin was injected in extremely large doses the results indicated a high degree of resistance.

In the study of type C both in this country and in South Africa the susceptibility of a number of mammals has been demonstrated. Among those commonly used in experimental work are white mice, guinea pigs, and rabbits, of which the first are the most resistant. In South Africa, goats, sheep, and cattle, which come in contact with lamsiekte under natural conditions, were found to be particularly susceptible.[12] Horses, mules, and donkeys were more tolerant of the poison, while rats, dogs, and pigs displayed a resistance that approached, if it did not actually reach, complete immunity. Essentially the same results were attained by Graham and Boughton (*26*) in this country with such of these mammals as they had used, namely, guinea pigs, rabbits, dogs, cattle, and horses. Of particular interest are the reactions of monkeys to the toxin of type C *botulinum*. Gunnison and Meyer (*32*) found that these mammals are resistant to large doses of both types C and D toxin when given by mouth, though they are susceptible to injected doses.

Recently Gunnison and Coleman (*29*) have shown that, "while small laboratory animals were highly susceptible to the toxin of the duck strain when injected subcutaneously, they were remarkably resistant when it was administered by mouth." They also brought out

[12] Comments on the susceptibility of mammals in experimental work in South Africa are based on work done before types C and D had been differentiated.

the fact that the ratio of the injected to the oral minimum lethal dose was, for guinea pigs, 1:1,800; for mice, 1:22,000; and for rabbits, 1:14,800.

HUMAN BEINGS AND TYPE C BOTULISM

When duck sickness first attracted national attention, there was considerable apprehension as to the possibility of human infection. In the absence of a satisfactory diagnosis of the malady and in view of the wide-spread use of wild fowl as food, this fear was by no means without reason. Some hesitated to handle the sick and dead birds, and there were rumors of the contraction of the disease by people. That was in the days when the alleged causes of the malady ranged from typhoid, through the whole gamut of avian diseases, to direct intoxication by arsenic or other poisons from industrial waste. Even persons inclined to meet such emergencies without hysteria urged disinfection of ducking grounds, and this led to the practice of burying the dead bodies or covering them with lime (p. 33). Some duck clubs were closed during seasons of severe outbreak, but this was prompted not alone by a feeling that there were dangers involved through the human consumption of the flesh of affected birds, some of which still could fly, but also by a genuine desire to conserve the remaining birds.

There is no question that many ducks affected with duck sickness at the time they were killed have been eaten by human beings. Many birds also were being picked up accidentally by hunters who mistook those killed by disease for those they had shot. In the days of market hunting in California, it was common knowledge that unscrupulous hunters made it a practice to gather birds affected with or recently dying of the disease, fire lead shot into their bodies, and offer them for sale.

This study has revealed no instance of human beings contracting botulism through eating the flesh of bird victims of duck sickness. In considering the susceptibility of man to type C botulism this fact is indeed significant. Although one would have to presuppose an adequate toxin-producing period in the flesh eaten and the absence of heat great enough to destroy toxin or the organism itself, many prefer their game rare and a bit "high", a preference that might easily lead to serious consequences were type C *botulinum* toxic to man. These circumstances lend further credence to the belief that types A and B *botulinum* are seldom causative factors in epizootics of duck sickness under natural conditions (p. 61). Further circumstantial evidence of the relative if not absolute immunity of man to oral doses of the toxin of type C, and possibly the closely associated type D, comes from South Africa, where in areas known to be infected with lamsiekte, the natives often may be found feasting on meat that is none too fresh.

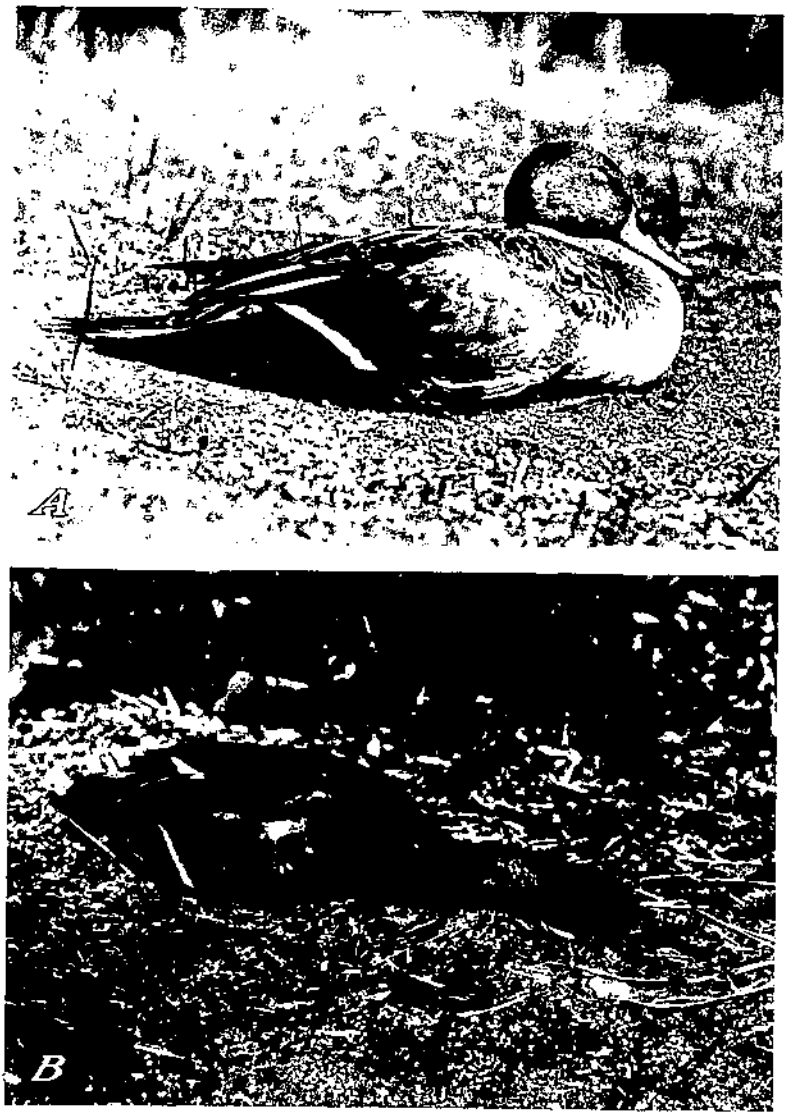
Recent experimental work by Gunnison and Meyer with type C botulism on monkeys (*Macacus rhesus*), from which analogies may be drawn with respect to its action on man, indicates that "they are resistant to large amounts of types C_α [= C of Bengtson] and D toxins when given by mouth, although readily intoxicated when injected subcutaneously" (*30*, *32*).

SYMPTOMATOLOGY

The general clinical picture of duck sickness (botulism) is one of extreme muscular weakness, brought about by an involvement of nerve centers or terminals (*18*). In wild birds this early results in inability to fly and then to walk, followed later by loss of muscular control of the neck, a paralysis, in many cases, of the muscles controlling the nictitating membrane, as well as those affecting pulmonary action, and, in the end, utter prostration, with death ensuing usually without struggle or apparent pain. A diarrhea marks some cases, followed often by an obstruction of the vent by hardened renal matter. Subnormal body temperature is a common symptom. A high percentage of recovery also results when birds are rescued from infected environments and afforded dry quarters, wholesome food and water, and some individual attention.

In duck sickness, as with most other intoxications, the rapidity of onset and the severity and even character of the syndrome are dependent upon the quantity of toxin ingested. Stating the case somewhat differently Theiler, writing of the analogous malady lamsiekte in South Africa, said: "It is possible to produce almost any clinical form of the disease at will by adjusting the dose of toxin" (*66, p. 1116*). Although a certain period must elapse between the time of ingestion and the manifestation of the initial symptoms, this may vary in birds from less than 2 hours (in the case of an experimental pigeon that had received an excessive subcutaneous dose) (*66, p. 1291*) to as much as several days or a week, when the affliction is mild and followed by recovery. Such differences in the quantity of toxin assimilated produce cases that may be acute or subacute, and when a paralysis or weakness persists after partial recovery, the disease may acquire even a chronic aspect. Among wild ducks subacute cases predominate, and acute ones are not uncommon. Those that may be considered chronic are rare and usually run into complications that tend to obscure the immediate cause of death. In describing in detail the nature and sequence of symptoms, a subacute, sublethal case will first be discussed.

Prior to the discovery of the cause of duck sickness and the means for infecting experimental birds, some of the early symptoms could not be observed for the reason that wild birds so affected could fly or otherwise evade capture. In the case of a mallard that has ingested a quantity of toxin somewhat less than lethal, one may expect to see the first uncertain symptoms about 24 hours after ingestion. The bird has a tendency to rock forward, throwing its weight onto the toes. At this time it still can use its wings vigorously, even though unable to maintain prolonged flight. The slight uncertainty of equilibrium soon is aggravated and followed by an unsteadiness on the feet, revealed as a halting gait and an inclination not to move. Later the bird assumes a straddle-legged position while standing, and its efforts to walk are confined to short, hesitant steps in which is revealed for the first time the physical weakness invading its system. Even prior to the appearance of the first noticeable symptoms afflicted birds refuse food and water, a circumstance that readily explains the almost total absence of food in the stomachs of birds that die of duck sickness.



(Over)

A, Sick pintail, unable to fly or walk, inclined to rest its bill on the crook of the neck, as nictitating membrane paralyzed. *B*, Mallard in the final stages of duck disease. Birds so affected are utterly prostrate, respiration is slow and feeble, and the heart action weak.

A, Sick [illegible] in which the neck muscles are partially paralyzed, resulting in the [illegible] shown in the neck of this bird. B, Viscera of [illegible] bird, showing distention of [illegible] lower intestine caused by [illegible]

At this point, about 30 hours after ingestion of the toxin, ability to fly is noticeably impaired, and the bird may not be able to rise from the water. It still can make rapid progress, however, by combined wing and leg action. The body, partly lifted from the water, is dragged over the surface by noisy wing beats. As soon as cover is reached the bird seeks to conceal itself and conserve its declining strength. It still may be able to stand, but its tendency to squat increases, and in doing this it evidences a pronounced weakening of muscles controlling the tibio-tarsal joint. The bird may lower its body normally for a portion of the distance but then suddenly loses control and drops to the ground. Paralysis soon begins to affect some of the muscles controlling the wing, including those that support it when folded next to the body. These relax, causing the "wrist", or carpal, joint to lower, and at times there is a complete wing droop, the primaries touching the ground.

Even after ability to stand or to move the wings is lost, affected ducks still can swim, and when they encounter shallow water or mud flats, they manage to make feeble and uncertain progress by thrusting themselves forward on the breast. In this stage some ducks, particularly redheads, attempt to evade capture by diving, if in deep water. At times, however, redheads and, more frequently, other ducks have difficulty in submerging, owing possibly to their inability to deflate certain air sacs. Birds so affected may be found with the head beneath the surface, paddling more or less vigorously, but making no downward progress.

A number of symptoms more or less closely related to the spread of paralysis to regions of the neck and head appear at about this stage of the syndrome. For some time the bird has been holding its head with the bill resting on the crook in its neck (pl. 3, *A*), but as the victim weakens, inability to maintain an upright posture of neck and head increases, and from time to time the head will pitch forward until the tip of the bill touches the ground, or it will drop to one side or the other, where it may rest for short periods sidewise, or sometimes in an almost inverted position.

Even previous to the arrival of this condition one may detect in many cases a paralysis of the nictitating membrane, that "third eyelid", which in healthy birds flashes backward from the anterior corner of the eye on the slightest irritation. At first this affliction may be indicated merely by a certain sluggishness in the activity of the membrane, but later the bird is unable to draw it across the entire width of the pupil. This results in the drying of the unmoistened portion, where particles of dust soon gather, and often a sharply defined line marks the edge of the area over which the nictitating membrane still functions. Finally the third eyelid becomes wholly inactive and lies inert and concealed at the anterior corner of the eye.

Paralysis of the nictitating membrane, though a common manifestation of duck sickness, is by no means a constant or diagnostic symptom. Geiger, Dickson, and Meyer (*24*) have stated the case properly with respect to barnyard fowl afflicted with limberneck when they said, "*as a rule*, the movements of the nictitating membrane are sluggish . . ." When this symptom appears it may be considered characteristic of the earlier part of the syndrome. In

fatal cases it may persist until the end; in mild ones it may be apparent for only a short period of the bird's disability or, as was demonstrated by experiment, it may not appear at all, even though paralytic conditions exist in other parts of the body. Among birds recovering from duck sickness it is common to have the normal activity of the nictitating membrane restored before full control of legs or wings is regained, and to find this membrane functioning perfectly even in helpless birds whose outer eyelids are completely sealed by hardened exudates. In the course of this investigation, beginning with the observations of Sperry in 1927, individuals that did not reveal a paralysis of the nictitating membrane have been encountered in the field, including the following species: Great blue heron, black-crowned night heron, pintail, coot, killdeer, avocet, ring-billed and California gulls, and yellow-headed, red-winged, and Brewer's blackbirds. Probably at least a portion of these were birds in which the function of the membrane had been restored after earlier impairment.

On the other hand let it be emphasized again that immobility or sluggishness of the nictitating membrane may occur in cases of saline intoxication (p. 21), and hence the symptom cannot be looked upon as diagnostic of duck sickness.

At this point mention of another symptom associated with the eye may be made by quoting directly from Wetmore (*71, p. 9*), who has fully described this aspect of the syndrome:

> Lying within the orbit on the anterior surface of the eyeball is a large gland, known as the Harderian gland. This secretes a fluid that reaches the eyeball at its anterior corner below the nictitating membrane. In the duck sickness this gland always seems more or less affected and in most cases is considerably swollen, so much so, in fact, that the eyes are protuberant. Following this swelling the discharge of colorless, watery fluid from the gland becomes more copious. Normally it escapes at the anterior corner of the eye through two canals that unite and lead into the nasal chamber. In sick birds the secretion becomes greatly augmented, however, until these openings are not able to care for it, the eyes appear watery, and the fluid escapes between the lids. In some pintails kept under observation the escape of this fluid moistened the feathers of the entire side of the head. In a few hours this discharge becomes viscous and more or less opaque, and cements together the eyelids, while the augmenting supply held within puffs out the lids all around. The portion that escapes through the ducts passes through the inner nasal openings into the mouth. As the opening into the trachea (the glottis) lies immediately below, the fluid clogs it and interferes greatly with breathing. After two or three days the secretion becomes caked and cheeselike. When it thickens in large quantity it sometimes closes the trachea and causes strangulation. In a few cases the fluid penetrated to the bronchi, filling them completely and killing the bird.

Coincidentally with or even prior to the paralysis of the nictitating membrane a dyspnea, or air hunger, may appear. Often the lower mandible drops slightly, and respiration becomes a series of gasps in which the head is raised with each inspiration. This labored breathing, visible at a considerable distance, served as a diagnostic recognition mark in the search for sick birds in the field. It is caused no doubt by impairment of the muscles controlling pulmonary action, coupled in some cases with an obstruction of the glottis, or trachea, as just described.

A greenish diarrhea develops in many ducks between 24 and 48 hours after ingestion of the toxin. At times this is pronounced,

and if the bird has lost its ability to move about, the plumage of the underparts becomes greatly soiled. Here again, as with the paralysis of the nictitating membrane, the symptom covers only part of the period of the bird's disability. After a day or two of profuse watery discharge the white renal matter of the feces increases and tends to harden, resulting, at times, in a complete stoppage of the vent (pl. 4, *B*). In addition to a more or less mechanical obstruction, there may be at this stage an inhibition of gastro-intestinal movements much as in human cases of botulism, in which constipation is a characteristic symptom.

The peak of the syndrome, in cases just sublethal in intensity, usually occurs between 48 and 60 hours after ingestion of the toxin. At that time the bird may be completely prostrate, with the neck outstretched, wings relaxed, and feet beneath it or extending backward (pl. 3, *B*). The presence of life itself may be evidenced only by the rise and fall of the feathers of the back, with the slow and feeble respiration, which, as has been shown by Wetmore (*71, p. 8*) in a mallard, may be as slow as 8 per minute. At the peak of sublethal cases in pintails a respiration rate of 15 to 18 a minute is common. Heart action likewise is enfeebled and retarded, a pulse of 60 to 80 being recorded in a duck instead of the normal of about 120 per minute. Great irregularity in the pulse also is often encountered. This has ranged from 60 per minute when the bird was at rest, to a speed too fast to compute when the victim has been endeavoring to escape capture. Body temperatures are quite uniformly subnormal in all severe cases. From a normal of 104° to 109.8° F. (mean 106.7°) (*71, p. 8*), rectal temperatures of prostrate ducks often may be as low as 100° F., and, when death is near, the body may even feel cool to the touch. In a mallard that was prostrate after ingestion of a lethal dose of toxin the rectal temperature was below the lowest graduation (94°) of the clinical thermometer used.

Signs of recovery in a duck that has ingested a dose slightly less than lethal may be expected on the third or fourth day. If the bird has been prostrate, ability to move and raise the head marks the turn for the better. If the eyelids have been sealed by exudates the bird will reveal its first active effort to regain normal conditions by rubbing these on its back, first on one side and then the other. If water is placed before it, the bird will drink large quantities at this early stage of recovery, even though it may still be unable to see. In doing so it is likely to choke, apparently as a result of a continued impairment of the pharyngeal muscles.

On the fourth or fifth day the bird will preen its feathers and may even rise to its feet. It gives great attention to restoring its plumage to a normal water-shedding condition. Muscular weakness still persists, however, and there may be repeated hitching of the wings in an effort to hold them properly at the sides.

On the fifth or sixth day one may expect to find the bird walking haltingly and even stretching or flapping its wings. Coincidentally, for the first time since its affliction, it will again take food. A day or two more and it will have regained strength sufficient to fly short distances and be able to care for itself when released.

Such is the usual sequence of symptoms in a severe but sublethal case. Greater quantities of toxin will result in a more rapid and

violent syndrome, and often in an inversion of the usual order of some of the symptoms. Occasionally a duck with complete lack of control of head and neck, still retains ability to travel across the water by lusty wing beats; this may cause the head to flop from side to side, and, when progress stops, it may drop beneath the surface of the water. At times nerve impairment may cause a paralysis that is not symmetrical with respect to the two sides of the body. One affected ring-billed gull could stand only if placed with its left side against the wall of its cage; on the open floor it invariably fell to the left. When directly antagonistic muscles are involved, an unequal paralysis may upset the coordinating mechanism and cause a pronounced torticollis. Such a condition is not infrequent among gulls and avocets (pl. 4, *A*). Doses of toxin so excessive as to cause death within a few hours may produce convulsive reactions, whereas in more protracted cases, the victim at no time appears to be in pain other than that incident to difficult breathing. Avocets and stilts become quite helpless when the paralysis affects their long legs. These often become entangled in masses of aquatic vegetation, from which the victims seldom free themselves. Air hunger is a more pronounced early symptom among gulls than ducks. Both gulls and coots may exhibit complete control over the muscles of the head and neck, even to the extent of being aggressively pugnacious, while at the same time their legs and wings are helpless. One ring-billed gull could peck vigorously at a time when its nictitating membrane was completely paralyzed.

Loss of feathers or a looseness of plumage, considered to be of some value in the diagnosis of limberneck in domestic poultry, was not found to be a common attendant factor among wild ducks. Now and then an individual duck would lose many feathers during its period of disability, but part of this at least could be associated with the post-eclipse molt.

A persistent paralysis was noted among some experimental birds after they had recovered from the principal effects of the disease. This was manifested usually by inability to hold the wing properly in place. Occasionally a leg weakness developed that was retained for a week or two after the bird had resumed feeding and other activities. The victims of such chronic disabilities as a rule became much emaciated, took little care of their plumage, and seldom effected complete recovery.

It is evident from field observations that few of the dead birds encountered had experienced the violent symptoms indicated in the experimental work with excessive doses of toxin. Almost invariably dead birds are found lying on the breast, with plumage unruffled and unsoiled except at the vent. Death comes slowly, without struggle, and apparently without pain.

PATHOLOGY

Birds that succumb to duck sickness are, as a rule, in good flesh, a condition to be expected from the fact that in most fatal cases the period of sickness is relatively brief. Only those that have undergone the ordeal of protracted affliction or have had successive experiences with the malady show appreciable emaciation.

The alimentary tract usually is devoid of food, the gizzard containing merely gravel or the hard, indigestible parts of earlier meals. This state of affairs long has tended to conceal the food items or the nature of the feeding environment directly connected with the contraction of the malady.

Though nothing of pathological significance has been found in the gullet or proventriculus, Wetmore has pointed to a sloughing of the stomach lining at the lower edge of the proventriculus. This condition was present in birds succumbing at Great Salt Lake, though not confirmed with any degree of regularity among birds of the Klamath Falls district. Irritation and congestion of blood vessels of the intestine, though frequent, was by no means constant among birds in southern Oregon and northern California. The small intestine, however, was shrunken and firm to the touch and often contained straw-colored mucoid matter. Graham and Boughton (*36*), discussing the pathology of limberneck, have referred to "petechial hemorrhages and areas of congestion and catarrhal inflammation of the mucosa of the small intestines", and Robinson (*56*) has noted "evidence of catarrhal gastro-enteritis" in a few cases of avian botulism in South Africa.

The larger intestine below the caeca often presents a striking condition that in the case of avocets and gulls becomes almost diagnostic. This consists of an obstruction of the vent due either to the hardening of the renal matter deposited there or to the impairment of muscles controlling the vent, with a resultant accumulation of excretory matter that distends the cloaca often to enormous proportions (pl. 4, *B*). The contents are largely liquid, with varying quantities of granular renal matter.

Other organs of the body appear normal or nearly so. The ventricles of the heart are found in systole. The gall bladder usually is filled and occasionally distended. The lungs, liver, spleen, and kidneys reveal nothing of pathognomonic significance. An erythrocyte count of the blood of sick and healthy birds likewise disclosed no condition of diagnostic value. There appeared, however, an increase of erythrocytes in sick birds, a condition that may possibly have arisen merely from failure to drink for several days.

Dickson has reported that it is common to find hemorrhages in the central nervous system in human beings and experimental animals (*13*, *14*). Engorgement of the blood vessels of the meninges of birds dying of duck sickness, particularly in severe or protracted cases, frequently is revealed at necropsy. Though the hyperemic areas are variable in extent, they are, as a rule, restricted to the surfaces of the medulla, ocular lobes, and posterior half of the cerebrum. Others who have sought for pathognomonic lesions in botulism have called attention to the frequency of disturbances in the central nervous system and its meninges, but as yet nothing that can be considered absolutely specific in character has been detected.

Cowdry and Nicholson (*11*, *p. 840*), after an exhaustive study of the central nervous system in botulism, stated that—

> . . . except for a slight degree of vascular engorgement, all the lesions which we have noted in the brains of mice, guinea pigs, and rabbits suffering from *botulinus* poisoning are readily susceptible of some explanation other than that they are produced by the direct action of the toxin upon the central nervous system.

Dickson, who formerly called attention to apparently diagnostic thrombi in the central nervous system, later stated (*16, p. 77*): "Gross and histologic examinations of the tissues of an animal which has succumbed to botulism fail to show anything that can be considered diagnostic."

If anything may be considered diagnostic of the gross pathology of duck sickness it is the negative evidence created by the lack of any constant or characteristic lesion. In the majority of necropses, however, one may expect to find a certain engorgement of intestinal blood vessels, a hardening of the small intestine, a yellowish mucoid intestinal content, a stoppage of the vent with a resultant distention of the cloaca, and a congested condition in the meninges of the posterior half of the brain.

DEMONSTRATION OF BOTULISM AS THE CAUSE OF DUCK SICKNESS [29]

BIRDS USED IN EXPERIMENTS

During 1929 and 1930 much of the experimental work on duck sickness was conducted with wild ducks, gulls, and coots trapped in the Klamath Falls district. When the disease finally appeared in the field and sick birds were being rescued a certain portion of those that recovered were used. During the winter of 1930–31, however, domestic pigeons were employed in experimental work at Washington, D.C., and the satisfactory results attained prompted their continued use during field work in the summer of 1931 and in subsequent experimentation at the University of Minnesota.

Although the use of pigeons in experimental work in botulism had no extensive precedent, it was found, at least when toxin was injected intraperitoneally, that these birds met all essential requirements. Not only are they susceptible to relatively small doses (as little as 0.0001 cc of type C filtered toxin killing in 18 hours in one instance) but they also respond well to toxin-antitoxin tests and reveal a typical and recognizable syndrome. Furthermore, the laboratory use of pigeons had the practical merit of saving valuable wild fowl.

Justification of the use of pigeons in experimental work in botulism has been established by Gunderson in the course of this study. He has shown that—

> mixed (impure) cultures from various sources are ordinarily incapable of causing the death of a pigeon unless *Clostridium botulinum* is present. In one series of experiments involving the injection of materials, either as enrichment cultures or directly from the field, only 4 of the 352 pigeons used died from causes other than botulism. To make the data even more convincing, record of other birds could be added—those surviving toxin-antitoxin injections and those used in subsequent experiments.

Gunderson, using a double series of cultures, one in ordinary broth and the other in the alkaline medium employed in studies of botulism, tested the susceptibility of pigeons to 12 known organisms that might appear as contaminants. A 0.5-cc dose was injected, and only one culture (that of *Bacillus proteus* in ordinary broth) produced ill effects, the pigeon dying with lesions of peritonitis. In subsequent experiments conducted at the University of Minnesota it was

[29] The information here presented has been derived from experiments conducted by Kalmbach in 1929–30, and from data contributed by Gunderson in 1931 and 1932, when facilities for more thorough and accurate bacteriological work were available (*28, 41, 42*).

demonstrated by Gunderson that, whenever *B. welchii* appears as a contaminant, disturbing results are likely to follow. Despite these reactions the uniformly satisfactory results attained in other experiments seem fully to justify the use of pigeons for studying this avian aspect of botulism.

In toxin-antitoxin tests, injections were made into the body cavity directly beneath the posterior border of the sternum. Unfiltered cultures or extracts being tested were injected in 0.5-cc dosages. It was found that when such large doses were employed, symptoms became evident within 36 hours if toxin was present, the usual period being about 12 hours. It should be pointed out, however, that the death of an experimental bird was not accepted as evidence of botulism even though the symptoms pointed to it. Each culture considered to be positive was accepted as such only when toxin-antitoxin experiments confirmed the diagnosis.

Pigeons surviving the injection of cultures could be used again satisfactorily after a lapse of time; but the use of birds that had received antitoxin was considered unwise because the possible creation of an immunity, or the possible presence of residual, uncombined antitoxin. Actual experimentation has indicated that residual antitoxin may be encountered in cases where toxin has been overneutralized and injections made at intervals of from 7 to 10 days.

Serum sensitivity also has been observed in birds receiving antitoxin. In one experiment 7 of 15 previously used birds died of anaphylaxis upon subsequent administration of antitoxin. Others also have noted such a reaction (*23*). Likewise immediate death due to altered antitoxin has been noted in two instances both involving type B antitoxin. That the antitoxin was incriminated is indicated by the fact that substitution of a fresh lot caused this difficulty to disappear (see also comparable results of Topley and Wilson, *68*). Even the injection of cultures alone has produced immediate death in pigeons, with symptoms simulating anaphylaxis, a reaction observed by Hewlett, Bullock, and O'Brien (*36*).

Most of the antitoxins used in this work were obtained from a commercial firm. All type A antitoxin from that source contained antibodies against type C toxin, while those for types B and C were specific.

REPRODUCTION OF THE SYMPTOMS

The reproduction of the syndrome of duck sickness in experimental birds marked the initial forward step in diagnosis of this malady. This was accomplished (1) by the feeding of incubated body tissues of birds that had died of the disease; then (2) by the injection of normal saline extracts of such toxic material; and, still later (3), by the feeding or injection of standard culture media previously inoculated with material from duck-sickness sources. Opportunity was afforded to note the similarity of symptoms in birds made sick in this manner with those of birds found affected in the field. Cultures of known type C *botulinum*, obtained from a reliable source, likewise produced symptoms typical of duck sickness.

During the latter part of the 1930 field season and in certain experiments conducted during the following winter, incubated body tissues (mainly livers) of birds that had died of duck sickness fur-

nished a convenient source of toxin for experimental work and at the same time afforded evidence of the invasion of body tissues of sick birds by the organism. The material was prepared first by forcing the tissues through the mesh of ordinary pearl-netting fly screen, thus producing a gruellike mass that could readily be measured or weighed. This was then placed for 5 or more days in an incubator, where a temperature of between 85° and 95° F. was maintained. No pains were taken at that time to create an anaerobic condition during incubation. Doses were administered in gelatine capsules, and though crude in technic and involving the use of highly impure cultures, this method proved adequate for the early bacteriological work; later (1931) it was superseded by more approved methods, in which standard culture media and a procedure better suited to the growth of anaerobic bacteria were adopted.

It is fitting to mention a single experiment performed by Sperry in 1927. At that time the idea that duck sickness was in fact botulism, had not been advanced, but an attempt was being made to transmit the mysterious malady by feeding healthy ducks the livers of birds that had died of the disease. A mallard was force-fed a small quantity (about 4 cc) of such tissues daily. At the end of 3 weeks the bird suddenly developed typical symptoms of duck sickness and ultimately died. Today, with a better understanding of the malady, we may surmise what happened in that early experiment: The tissues originally were fresh or nearly so. At some point, however, near the end of the series of doses, the liver used had been kept long enough for the toxin of *Clostridium botulinum* to be formed in quantity sufficient to kill. Although the results of this experiment remained unexplained at that time and for 3 years following, there is now no doubt that Sperry produced true duck sickness in that early experiment.

The largest single group of tissue-feeding experiments in this investigation was that involving the administration of incubated livers to ducks. Forty-three individual experiments of this character were performed, in which 93 birds were used, mainly pintails and mallards. In 14 of the 43 experiments no toxicity was demonstrated, negative results usually being associated with certain lots of incubated liver in which apparently the *botulinum* toxin had not developed. Among the others the results varied from cases of rapid intoxication, with death following in less than 6 hours after ingestion of the toxin, to sublethal cases, in which all typical symptoms were revealed, followed in due course by complete recovery.

The degree and rapidity of intoxication conformed closely to the size of the dose. This was well illustrated in one experiment in which five female mallards of nearly equal weight were given oral doses of toxic liver of 1, 2, 3, 4, and 5 g, respectively. Although all the cases resulted fatally and the onset of symptoms was rapid, they were graduated in intensity almost in direct proportion to the quantity of toxin ingested. Comparable reactions were detected when equal doses were fed to birds of different body weights.

That internal organs, other than livers, of birds dying of the disease may become the source of toxin when properly incubated was brought out in experiments in which typical duck-sickness symptoms were produced by the feeding of incubated kidneys. In

one of two experiments positive results were obtained also by the use of the incubated blood of birds that died of the disease.

With an equal degree of uniformity the syndrome of duck sickness was produced by the intraperitoneal injection of saline-solution extracts of toxic tissues. In this procedure a small quantity, usually 1 to 3 g of minced toxic tissue, was agitated in a somewhat greater volume (5 to 10 cc) of saline solution and allowed to settle, after which the supernatant liquid was further diluted to reduce the concentration to a point where the small doses needed for injection could be conveniently measured. By this means the susceptibility of certain birds, highly refractory to oral doses, was demonstrated (pp. 44 to 45). The results with gulls were of particular interest, since these birds develop certain peculiarities of syndrome that aid in revealing the similarity of the disease produced in the laboratory with that found in the field. Further confirmation of the identity of the experimentally produced disease came to light on many occasions in later work (1931–32), when standard cultures and filtered toxin, originating from material associated with the disease in the field, took the place of incubated body tissues or their extracts.

The clinical picture of this experimentally produced disease, as well as that of duck sickness in the field, is similar to that of avian botulism as it has been recorded in literature. Opportunity to make comparisons with the syndrome produced by the toxin of known type C botulism was afforded in numerous experiments involving both ducks and pigeons. Ofttimes sick birds from the field, those made sick with cultures originating from field sources, and those inoculated with known type C botulism were on hand at the same time.

RECOVERY OF THE ORGANISM

Recovery of *Clostridium botulinum*, type C, from the internal organs of a bird that has died of duck sickness is not generally looked upon as having diagnostic significance. This is particularly the case when recovery is from a point in the alimentary tract, which often may contain a few spores or vegetative forms which, under these conditions, are harmless and possess nothing of pathogenic importance. Gunderson has shown that in a series of 10 pigeons fed lethal doses of type C culture, 8 revealed the presence of the organism in the liver, which had been removed aseptically immediately after death. The internal organs (mainly livers) of a series of ducks, geese, and shore birds that had died of duck sickness in the Klamath Falls district and in other disease-infected areas of the West disclosed type C *botulinum* in 62.9 percent of the samples.

In distinction from this relatively high frequency of recovery from the tissues of birds dying of the disease is the infrequency of the organism in the tissues of birds dying from causes other than botulism. Gunderson has demonstrated that the cultured livers of 20 unaffected pigeons failed to disclose the organism in a single instance. In commenting on his examination of ducks that had died of lead poisoning in Minnesota, he reports that in a series of cultures of 34 birds, only 1 (2.9 percent) revealed type C.

It is of more than ordinary interest to note that type A has been recovered from the livers of ducks dying of lead poisoning (2 cases) and from the liver of an apparently healthy ruffed grouse (1 case).

Hobmaier (*40, p. 14*) likewise has stated that he has demonstrated the causative organism of duck sickness in the livers of 22 of 24 birds dying of the disease, whereas "47 investigations of birds not having suffered from the sickness failed to show this organism."

The initial determination of *Clostridium botulinum*, type C, from tissues of birds dying of duck sickness was made by Giltner as early as October 3, 1930. At that time the organism was detected in the internal organs (mainly the livers) of wild mallards, pintails, and ring-billed gulls, as well as in mud from Tule Lake. Since that time the organism has been recovered from the internal organs of a number of other wild species. Later Giltner found it in the liver of a juvenile prairie falcon, collected at Tule Lake, and in the work conducted at Klamath Falls, it was recovered by Gunderson from the tissues of the following species: Canada goose, green-winged teal, coot, long-billed dowitcher, western sandpiper, avocet, northern phalarope, California gull, and Bonaparte's gull.

In addition to the material gathered in the Klamath Falls-Tule Lake area, *Clostridium botulinum*, type C, has been identified in the internal organs of wild birds collected on the Bear River marshes, Great Salt Lake, Utah; American Falls Reservoir, Idaho; Colusa, in the Sacramento Valley of California; the mouth of the New River at the south end of the Salton Sea, Calif.; Cone, Tex.; Antioch, Nebr.; Antelope Lake, Clark County, S.Dak.; and Windom, Cottonwood County, Minn.

Such circumstantial yet significant bacterial evidence, gathered from widely scattered areas of infection, adds weight to the already well-established hypothesis that duck sickness is the same throughout its range.

The causative organism has been found in many media other than the bodies of birds dying of the disease. Though this has no significance from a diagnostic standpoint, it gives an idea as to the location of the organism in the field. Positive results were obtained on numerous occasions with soil or mud on duck-sickness areas. Often such material was heavily laden with miscellaneous organic matter, including strands of filamentous algae, masses of living and dead *Lemna*, copepods, snails, and insects. Debris from the Bear River flats, composed almost entirely of insect remains, disclosed the organism. Likewise it was found in both the larvae and pupae of hydrophilid beetles, the cocoons of which studded the surface of the dense algal beds at Tule Lake. Colonies of copepods, sarcophagid larvae (dead and alive) found on or near the bodies of dead birds, and even water obtained from the vicinity of such materials disclosed the organism. In Minnesota, Gunderson found the causative organism in dead and living snails. At Tule Lake wheat heads of the season's crop, as well as barley and mixed grain (chick feed), when submerged for a time in the water of infected environments, frequently yielded *Clostridium botulinum*. At the mouth of Bear River the decaying tubers of bayonet grass (*Scirpus paludosus*) harbored the organism.

In a series of 96 cultures inoculated with material from various media collected in duck-sickness areas at several points in Western States, Gunderson has detected type C *botulinum* in 45.8 percent. The contaminating material was divided about equally between mud,

water, and shore debris on the one hand and various items of bird food on the other.

In studying the prevalence of the causative organism in the field Hobmaier (*40, p. 14*) has demonstrated its presence in 28 of 76 mud samples collected in suspected areas. He records it also in 2 of 8 samples of foam collected from the surface of ponds.

DEMONSTRATION OF TOXIN IN THE FIELD

After the syndrome of botulism had been reproduced by feeding or injecting toxic material originating from tissues of birds that had died of the disease, and after the type C *botulinum* had been recovered from numerous samples of pertinent material, there still remained, at the outset of studies in 1931, the necessity of demonstrating the presence, in the field, of preformed toxin in or associated with food items or water that might be ingested by birds. This was accomplished in more than a score of instances, thus completing the etiological picture in the demonstration of botulism as the cause of duck sickness.

To Gunderson is to be credited much of the success in this phase of the study. The technic employed involved direct intraperitoneal injection into pigeons of 0.5-cc doses when the medium was fluid. From more nearly solid media, toxin was first extracted with a normal saline solution, which was then injected. In no case, however, was there any preliminary incubation or culturing. Whenever toxicity was demonstrated and botulism was indicated, the type involved was determined by adequate toxin-antitoxin tests. It may be explained that in all material gathered in the Klamath Falls district only the type C *botulinum* was disclosed.

Gunderson has demonstrated that of 76 samples of suspected material collected in sickness areas at Tule Lake 22, or nearly 29 percent, disclosed the presence of *botulinum* toxin. In seeking for toxin in the field, search was directed first to the carcasses of birds that had died of the disease and in such media toxin demonstration was not difficult. This was accomplished usually with bodies found lying on exposed flats, but the process was aided at times by encircling submerged bodies with metal cylinders to prevent undue dispersal of toxin by water movement. Toxin of type C botulism has been so demonstrated in the bodies of western sandpipers, avocets, coots, pintails, and green-winged teal. It likewise has been revealed in solution in shallow water adjacent to such bodies.

The larvae of sarcophagid flies feeding on infected carcasses die in great numbers when unable to find places sufficiently dry for pupation. These larvae were shown to be the source of a potent toxin, even when wind and water action had carried them considerable distances from the place where they were reared. Such material easily may become an intoxicating food item for shore birds and ducks.

Even living sarcophagid larvae may be toxic, as was demonstrated in one experiment, in which 15 were removed from the body of a green-winged teal and fed to a healthy bird of the same species. This bird later developed typical duck-sickness symptoms and died 6 days after ingestion of the larvae.

Toxin associated with dead sarcophagid larvae was revealed in several experiments in which the liquid from crushed larvae or that

filtered from masses of them was injected into pigeons. Typical of these is one in which 0.5 cc of liquid derived from dead maggots, which had floated some distance away from the body of an avocet, where they were reared, killed a domestic pigeon in less than 24 hours after a direct intraperitoneal injection. Circumstantial evidence of the contraction of duck sickness by the ingestion of sarcophagid larvae under field conditions was brought to light through the stomach examination of afflicted birds. Both the senior author and Sperry have found the remains of a score or more of these larvae in the stomachs of sick green-winged teal at Tule Lake.

Mud on which a dead duck had lain, but from which it had been removed, retained toxin in substantial quantities for 17 days. This was demonstrated in an experiment in which 0.5 cc of a saline extract of this mud, when injected, killed a pigeon in less than 48 hours.

Toxin also was demonstrated in barley scattered in shallow water and on mud flats in infected areas. Liquid drained from such masses of grain as had lain exposed for a week proved lethal to a pigeon on intraperitoneal injection of a 1-cc dose.

The possibility of toxin production in natural media was further demonstrated by the incubation in the laboratory of material that was obtained from field sources. A mixed lot of insect debris associated with *Lemna*, algae, copepods, and snails produced toxin during an incubating period of 5 days. Relatively potent toxin was produced in the laboratory in grain that had been inoculated with the type C *botulinum* and submerged for 2 weeks in water, which, at the start, was highly alkaline. Such a reaction may explain the sudden outbreaks of duck sickness that at times occur on artificial ducking grounds where shallow-water areas are heavily baited.

The apparent toxicity of scattered grain also was revealed by field observations. On two occasions (late in August and early in September 1931) a marked increase in sick ducks was noted on overflowed areas east of Tule Lake several days after these had been baited heavily with barley. This was done with the definite idea of demonstrating the potential danger from submerged grain in infected areas. The toxin-producing properties of grain also were revealed in experiments in which mixed grains in open-top glass jars were submerged on infected mud flats and allowed to incubate for a week or more.

Hobmaier has indicated the toxin-producing possibilities of other natural media when properly incubated. These include beetles, grasshoppers, and the bodies of fish (*30, p. 13*).[14]

In view of the high percentage of recovery noted among wild birds that contract this disease under field conditions, it would appear that the quantity of toxin ingested is either small or its potency low. Gunderson has pointed out that, as a rule, there is an appreciable interval between seizure and death among wild birds, which indicates a relatively small dose. It is possible, as he has also suggested, that the ultimate intoxication may be the result of several additive doses no one of which would have affected the bird.

[14] Mention is also made of additional substantiating evidence disclosed in California by Hobmaier (*30*), who has demonstrated the presence of toxin of type C botulism in the blood stream of sick birds. This was accomplished by inoculating mice, by which means extremely small quantities of toxin are demonstrated.

SUMMARY OF EVIDENCE OF BOTULISM

1. The clinical picture of duck sickness, both with respect to symptoms and pathology, is identical with that produced in experimental birds that have ingested the toxin of pure cultures of type C *botulinum*.

2. The causative organism has been recovered not only from the tissues of birds dying of the disease but also from soil and other material collected in infected environments. Though the former does not carry with it certain diagnosis, it is significant that the organism is frequently present in the tissues of sick birds and absent from those that are healthy.

3. The demonstration of the toxin of type C *botulinum* in food and water commonly ingested by wild birds points to the channel through which the malady is contracted. This study has revealed toxin under field conditions in such bird-food items as sarcophagidfly larvae and miscellaneous insect debris (attractive to shore birds and ducks), submerged grain (eagerly sought by ducks), and bodies of birds that have died from the disease (a common food source for gulls and magpies). The fact that mud of infected areas rich in decaying organic matter also has been shown to be toxic may account for much of the mortality of puddling ducks and probing shore birds. Toxin demonstrated in the water of small pools is a source of danger to any birds that may come in direct contact with it.

4. The incidence, course, and disappearance of duck sickness in the field, conform to the influences of temperature and environmental factors in a highly suggestive manner. In other words the epizootiology of duck sickness is just what one would expect in an outbreak of botulism among wild fowl.

FACTORS RESPONSIBLE FOR DUCK SICKNESS OR INFLUENCING ITS DISTRIBUTION

Since duck sickness (botulism) is an intoxication, and not an infectious disease, it is contracted by wild fowl only through ingestion of toxin preformed in suitable media. For this reason the malady has its basis not so much in any inferior or predisposing condition of the bird as in factors affecting the welfare of a micro-organism living apart from it. These factors include such things as (1) prevalence of dead organic matter, (2) degree of alkalinity or salinity, (3) temperature, (4) water level and water movement, and (5) when the malady appears in epizootic intensity, an abundance of birds obtaining food or water in infected areas. It is a favorable aggregate of these factors that makes of duck sickness a seasonal malady reaching its peak late in August or early in September. It is the unfavorable reaction of one or more of these factors that may cause irregularity or even total absence of the disease in certain years, even though the organism itself may be abundantly present.

PRESENCE OF THE CAUSATIVE ORGANISM

The presence of the specific organism *Clostridium botulinum*, type C, is a prerequisite to the incidence of duck sickness in any locality. That it is widely distributed in the United States and is especially

abundant in areas where the disease has once occurred, there is little doubt. Meyer and Dubovsky (*47*) in the course of their studies of the prevalence of the human types A and B in this country encountered nontypical forms, some of which may have been the type C of Bengtson. Graham and Boughton (*26*) have recorded the organism at several points in Illinois, and Bengtson (*3*) has reported it in material from Missouri, Oklahoma, Illinois, and Maryland. This investigation has demonstrated its presence in such widely scattered areas as southern Oregon, northern and extreme southern California, the Salt Lake Valley, Utah, the Panhandle of Texas, western Nebraska, southwestern Minnesota, and the vicinity of Washington, D.C.

In the course of the study in Oregon and California Gunderson recovered the organism from 47.3 percent of 55 samples of mud, shore debris, and other material not generally looked upon as bird food. It was present in 44 percent of the 41 samples of material more or less intimately associated with bird food. In each instance the material was collected in a duck-sickness area.

In the course of later work in Minnesota, Gunderson recovered the type C organism from 4 of 11 samples collected in a duck-sickness area near Windom. This material included 2 lots of snails, 1 of maggots, and 1 of mud. In 99 samples from areas free of sickness, however, type C was disclosed only once and type A twice. In a study of 76 mud samples collected in supposedly infected areas in the Sacramento and Imperial Valleys during 1931, Hobmaier determined the presence of type C *botulinum* in 28 (*40, p. 14*).

The causative organism of duck sickness is now so prevalent and well established in the main centers of duck sickness that no theory of invading pollution is needed to explain any specific outbreak at these points. Its presence may be admitted, and the outbreaks explained on the basis of temporary conditions favorable to its growth and production of toxin.

PREVALENCE OF DEAD ORGANIC MATTER

Dead organic matter is necessary for growth and toxin elaboration by the causative organism of duck sickness. Such material is present in a great variety of forms in every marsh or mud flat area and on overflowed lands. Remains of insects, entomostracans of many kinds, mollusks, and even vertebrates (pl. 5, A) serve as excellent media for development of the organism. In one outbreak of botulism among captive ducks in South Africa, the bodies of earthworms were suspected of being the source (*56*). As brought out by this investigation, submerged grain and possibly other dead vegetable material may act as added sources of toxin, closely associated with attractive food items. Even finely divided and commingled particles of animal and vegetable matter found in the ooze beneath shallow water or on mud flats apparently play an important part in the intoxication of such birds as ducks and shore birds, whose puddling and probing for food throw them in direct contact with any toxin there formed. The peak of duck-sickness outbreaks coincides in general with the seasonal period of organic decay. Without this bacterial food supply the disease cannot gain headway, as

evidenced by the absence of duck sickness in waters overlying clean, sandy bottoms. This fact has served as the basis of the most effective remedial measure against the malady—the elimination of extensive mud-flat areas by proper water control (p. 73 to 74).

DEGREE OF ALKALINITY AND SALINITY

While much is yet to be learned concerning the effect of alkalinity on the growth and toxin production of *Clostridium botulinum*, type C, under field conditions (pl. 5, *B*) and possibly even on its distribution, there is no doubt concerning its important influence in laboratory experiments. Gunderson, citing other workers in this field, has called attention to the fact that the optimum range of hydrogen-ion concentration for growth and toxin production lies on the alkaline side of the scale. Bengtson (*3*) has stated that the medium should have a pH reaction of 8.0 to 8.5 for proteolytic and 7.0 to 7.5 for nonproteolytic strains of *C. botulinum* to insure the production of potent toxin.

By direct experimentation with a limited number of birds Gunderson has shown the favorable effect of alkalinity on the rate of toxin production by organisms originating from cases of duck sickness, and in later laboratory work he adopted a technic that guaranteed a definite alkaline reaction.

> In making the media, the formula of Bengtson (one part of chopped beef and two parts of water) was altered by substituting for the water a beef broth having a pH of 8.0, after which 10 grams of peptone were added per liter. Furthermore, a half-inch layer of calcium carbonate was placed in the bottoms of the culture tubes before the medium was introduced. The pH was greater than 8.0.

A condition of alkalinity prevailed in all waters directly connected with duck-sickness outbreaks that were investigated. This varied from a minimum pH reading of 7.1 (at Cone, Tex.) to a maximum in excess of 10.0 (in the Klamath Falls district), with an average of 8.5 based on 15 samples. A pH value in excess of 9.6 has been recorded for the open water of Tule Lake, although a reading of 9.4 or 9.2 is usual for the shallow water closer to areas of bacterial action on the shore. On the mud flats and in places where organic decay was manifest, the pH was lowered to 8.8 or less. It was quite apparent that the alkalinity of the lake itself was exerting a constant and pronounced neutralizing effect on the acidity resulting from bacterial action.

Gunderson also has called attention to the fact that—

> an alkaline pH, alkaline inorganic buffers, and a high concentration of various salts may partially sterilize the soil to the disadvantage of competing organisms, leaving the field to *Clostridium botulinum*.

It is also well established that despite the favorable reaction of a certain degree of alkalinity on bacterial growth and toxin production, continued or increased alkalinity or salinity has a destructive effect on preformed toxin. Thus, sick birds are not found in the immediate environment of highly saline waters, particularly at Great Salt Lake. That part of the shore line of this lake in direct contact with water having a saline concentration of about 20 percent has been free of sick birds, while the relatively fresh-water marshes at the mouths of affluent rivers were the scenes of disastrous out-

breaks. A similar condition exists at the Salton Sea, where sickness occurs, not in the highly saline environment of the open sea, but in the marshes and mud flats created near the mouths of the New and Alamo Rivers. Likewise Mono and Owens Lakes, Calif., whose chlorine-carbonate waters contain saline concentrations varying from 7 to 20 percent (*9, p. 162*), appear to be free of duck sickness, although they may harbor birds in goodly numbers.[15]

Duck sickness in epizootic proportions never has been reported on tidal flats washed by waters whose saline content approached that of the ocean (35,000 p.p.m.).[16]

In areas of highest salinity, the absence of duck sickness has always been a challenge to the validity of the theory of alkali toxicity. Under the new concept of duck sickness, this same challenge awaits explanation. For answer, one now looks to the bacteriological aspects.

Throughout the history of research in human botulism, beginning with the initial work of van Ermengem in Europe, the matter of growth-inhibiting or toxin-destroying agencies or reagents has been noted. Although most experimental work has been done with the human strains A and B, with the primary objective of preventing or curtailing toxin production in preserved foods, the results attained are significant, particularly when considered in connection with certain experiments of this investigation.

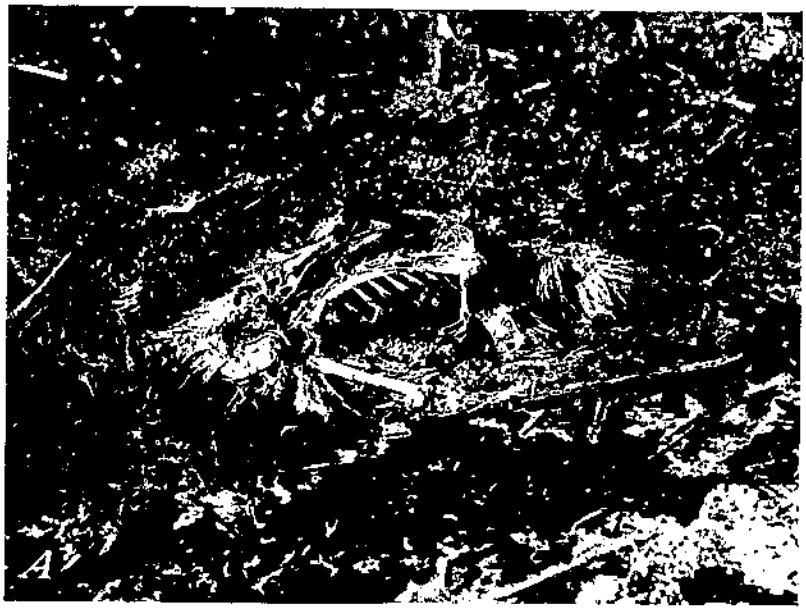
Van Ermengem himself, while working with what today is considered to have been type B, found that growth of the organism was inhibited in a medium containing 6 percent of sodium chloride (*19, 20*). Dozier (*17, p. 155*) found that "No strain of *B. botulinus* cultured gave any evidence of growth in double-strength veal infusion containing more than 8 percent of NaCl." Bidault (*4*) showed that 3 percent solutions of sodium chloride, potassium chloride, calcium chloride, and magnesium chloride nullified the toxin of type B and reduced the potency of type A. When a concentration of 6 percent of these salts was used, growth was completely inhibited. Thom, Edmondson, and Giltner (*67*), working on type A, found that toxin was produced in the presence of 5 percent but not 8 percent of sodium chloride. Wyant and Normington (*73*) found a somewhat greater tolerance to sodium chloride on the part of types with which they experimented. It is evident, however, that sodium chloride and certain other salts in concentrations as great as that of sea water tend to be unfavorable to the growth of types A and B.

Of even greater significance is the action of distinctly alkaline salts on preformed toxin. Here again the pioneer, van Ermengem, has pointed out that sodium carbonate—

> in the proportion of 0.5, 1, and 3 per cent, . . . when allowed to act on the toxin for 24 to 48 hours, rendered it innocuous to rabbits. . . . A solution of pure toxin in water was rendered instantly inert when an equal volume of 3 percent sodium carbonate was added (*3, p. 62*).

[15] An earlier report of bird mortality at Owens Lake (*22*), considered to have been duck sickness (*71*), was not, of course, substantiated by bacteriological evidence. It probably should be charged either to a lack of suitable food supply for birds that had just completed a long migration flight, or to direct saline intoxication.

[16] A possible case of botulism reported among shore birds on an island in Massachusetts Bay (*1*) may have had its origin in toxin produced in the carcasses of stranded blackfish (*Globicephala*) and a seal (*Phoca*), the bodies of which had been washed above high tide and therefore were out of direct contact with salt water (pp. 30–31).



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A, Decaying bodies of birds that have died of duck sickness become additional centers of infection. *B*, Crystalline deposits on shore-line debris alkaline salts that play an important role in the bacterial processes producing the toxin of botulism.

A

B

A, Field laboratory at Klamath Falls, Oreg., the scene of the Biological Survey's research work in 1929, 1930, and 1931. Nearby were pens for experimental and conv[illegible] birds. B, Resource affected birds, a means of saving many victims that otherwise would die on the road flats.

Other research workers also have demonstrated that the potency of preformed toxin is perceptibly reduced by an increase in alkalinity.

The toxin-destroying property of sodium carbonate was made the subject of experiments in this investigation involving both the feeding and injection of toxic material that had been subjected to the action of the carbonate (or in some cases, the bicarbonate) for variable periods. A 6-hour contact between a toxic extract and a 3-percent sodium carbonate solution completely nullified the toxin, and even a 1-percent solution in contact for 67 hours appreciably reduced its potency. A 0.5-percent solution, however, failed to effect a material loss in potency. With the bicarbonate of soda a certain retarding of toxic action was noted in one experiment, in which a 3-percent solution was in contact with the toxin for 24 hours.

In the light of this and other experimental work and the previously reported results attained by others with the chloride, it is of interest to note again the chemical composition of the waters of those lakes in which duck sickness has not been reported. Great Salt Lake (on the open waters of which the malady does not occur) has a sodium chloride content that fluctuates above and below 20 percent, depending on the depth of the lake; that of the Salton Sea (where the disease likewise is confined to contiguous, relatively fresh-water areas) is close to 3.5 percent (*10*). Sodium carbonate and sodium chloride form most of the 51,170 parts per million of total salts of the water of Mono Lake (*9, p. 162*). as they also do in the even more highly saline water of Owens Lake.

Although further evidence is needed, the foregoing indicates that in certain highly saline waters the absence of duck sickness may be due to a bacteriological factor—inability of *Clostridium botulinum* to thrive, or its toxin to persist, in the presence of high concentrations of certain salts. Even lower concentrations may be equally effective when the action is continuous. A seemingly anomalous situation is presented: Alkalinity in a pH sense is looked upon as a prerequisite or at least a most favorable condition for the growth of the causative organism and the evolvement of its toxin; yet, alkaline salts, in high concentrations, may reduce or completely destroy preformed toxin.

In view of the general conformity of the range of duck sickness to that of the region of alkaline waters in the United States (fig. 1); in view of the known favorable action of moderate alkalinity on the production of *botulinum* toxin; of the apparent buffering action of bodies of alkaline water on the acidity arising from bacterial action in decaying organic matter on shore lines or on mud flats; and in view of the possibility of such alkalinity destroying organisms competing with *Clostridium botulinum*, it would appear that moderate alkalinity is favorable to the type C organism and possibly a prerequisite to the epizootics among wild fowl. Furthermore, the deleterious effect of excessive alkalinity or salinity on the growth of this organism and on its toxin, facts recognized in the laboratory and indicated in the field by the absence of the disease in certain highly saline waters, points to a limiting factor worthy of mention. Alkalinity, therefore, may be a determining factor in the incidence of duck sickness in epizootic form.

TEMPERATURE

Temperature also plays an important part in the incidence of duck sickness, since it not only strongly affects the extent of toxin production by *Clostridium botulinum* but also influences the general processes of decay. These involve other bacteria, which, by reason of their consumption of oxygen, may aid greatly the growth of the anaerobic *Clostridium*. Although there is a wide range of temperature in which toxin may be evolved, Gunnison and Coleman have stated that the optimum for the growth of type C *botulinum* is 37° C. (98.5° F.) (*29*).

In general, the disease coincides with or immediately follows periods of hot weather. Lack of exact synchrony is to be expected, since various factors other than temperature control the incidence of the disease. Furthermore records based on atmospheric temperature are not always proper criteria on which to judge conditions where toxin is being produced. Beneath mud or water surfaces daily-temperature ranges are less extreme, and there one will find a seasonal variation that reaches a maximum in August and September, the period when outbreaks of duck sickness usually reach the peak. Ofttimes duck sickness stops rather suddenly when the temperature drops late in September or early in October. This, however, frequently may be due to the influx of irrigation water, or a change of bird movements with the advent of cooler weather, or to other attendant factors. As a rule outbreaks in central or southern California extend later into the fall than those in Oregon or Utah, and in the San Joaquin Valley in certain seasons the disease has persisted until December and January. The disastrous outbreak on the Bear River marshes in 1932, however, was severe late in October, and sick birds still could be found when ice had begun to form after the middle of November.

WATER LEVEL AND WATER MOVEMENT

Except for the requisite of ample organic matter for toxin production, no single factor enters more strongly into the etiology of duck sickness than the matter of water level and water movement. Even the less observant have noted that duck sickness is intimately correlated with areas of shallow, stagnant water and mud flats. Nothing will put an end to an outbreak more abruptly than a perceptible deepening of the water or a flushing of the infected area by heavy rains. Likewise, where water depths of a foot or more are maintained, the disease is seldom in epizootic form.

In the light of the present concept of duck sickness the reasons for such reactions are obvious. In the first place, deep water not only tends to keep temperatures down and thus retard toxin production, but the quantity of suitable media in the form of dead animal and vegetable matter will be less on deep bottoms than in shallow water or on shore lines, where the remains of surface life may be segregated en masse by wind and wave action. Likewise *botulinum* toxin is soluble and readily taken up, diluted, and dispersed by a large volume or movement of water. Furthermore, aquatic bird life, both shore birds and waterfowl, are particularly partial to shallow-water and mud-flat areas, where when not disturbed, they spend many hours in probing or puddling for food or just loafing and resting.

Circumstantial evidence of the important role played by shallow water is to be found in the relative infrequency of duck sickness among deep-water ducks, particularly canvasbacks, scaups, and ruddy ducks. Young redheads, however, do succumb in considerable numbers late in summer, when they feed extensively in shallow water, as also do pintails and mallards. Some mortality among diving ducks in spring has at times been charged to duck sickness. This has occurred in the Klamath Falls district as well as at Great Salt Lake, but the principal evidence as to identity is an apparent similarity of symptoms. There is the possibility that these deep-water feeders may reach spots of toxin elaborated during the previous season but not available to the shallow-water species.

Observations in the Klamath Falls district brought out the fact that duck sickness is by no means confined to conditions brought about by a recession or an evaporation of water. An equally dangerous state of affairs may arise from the creation of shallow-water or mud-flat areas by a slight rise or extension of water surface. The severe outbreak of 1925 at Tule Lake occurred after a marked rise in water, which submerged surrounding grainfields. In 1929 an area of infection was caused by the breaking of a low dike at the northwest corner of the lake, the water overflowing to adjacent wheat stubble. In 1930 and 1931 a slow rise in the water of the lake itself again produced extensive mud flats and shallow water to the east.

The influence of water level on the incidence of duck sickness also has been illustrated by the history of events at Great Salt Lake. The terrain immediately surrounding the lake is flat and, in years when the lake is high, the inflowing waters of the Bear River spread over wide areas. This happened in 1910, causing the most disastrous outbreak of record. Botulism in varied intensity followed a somewhat lowered lake level during the years immediately following. The next year of extensive mortality was 1921, when the seasonal peak of the lake level was fully a foot lower than in 1910, yet it was higher than in either of the two preceding years. From 1921 to 1931 some duck sickness occurred practically every year, but in 1929 a severe but short-lived outbreak in the immediate vicinity of the mouth of the Bear River ran its course at a time when the lake was relatively low but at a level that made this local area a stagnant mud flat. In December 1931, for the first time since 1907, Great Salt Lake dropped to the zero level of the Midlake gage, making the water in the area known as Willard Spur extremely shallow. In the summer and fall of 1932, wind action drove this shallow water over extensive flats, and a most disastrous outbreak of botulism followed (p. 34; pl. 1, *B*).

Incidents such as this, in which outbreaks of marked severity appear on newly flooded land that has been dry during periods of hot weather, lead to the surmise that, even though these lands may have been dry on the surface, conditions beneath may have been favorable to toxin production. With warmth, moisture, suitable organic matter, an alkaline environment, and a relatively anaerobic condition, subsurface toxin production might easily take place. The absence of flood water would tend to prevent toxin dispersal and dilution, and, when these areas are eventually submerged, the preformed toxin becomes readily accessible to puddling ducks.

ABUNDANCE OF BIRDS

An abundance of birds is a necessary corollary of a pronounced epizootic of duck sickness under natural conditions. Occasionally the infection becomes so intense in local spots that concentrated flocks may lose most of their numbers. Ordinarily, however, only a relatively small proportion of the birds frequenting a toxic area contract the malady. This would indicate that spots of toxin sufficiently potent to affect birds are probably very local.

Such a condition was demonstrated on overflowed land east of Tule Lake in 1930 and 1931. There, during August and September, a shallow-water area several square miles in extent cut by several low dikes was a constant attraction to a mixed flock of birds, mainly coots, pintails, mallards, cinnamon and green-winged teals, and Canada geese. Every morning this heterogeneous flock of 4,000 to 6,000 could be found in its customary place, feeding or loafing on the low dikes. A search over a circuitous route of about 2 miles would reveal about 20 sick birds. A similar search on the following day would reveal possibly only 8 or 10 newly sick birds; if the search were delayed a day or two an increase in the number of the newly sick and dead would be noted, but seldom was this number more than 25, representing about 1 out of every 200 birds present.

When large numbers of sick and dead birds are found during severe outbreaks, the mortality may represent the accumulated losses to successive migratory flights or feeding flocks that enter the locality unobserved at night. Although bodies of the dead rapidly disintegrate on the hot flats, marked evidence of the mortality may remain 10 days, 2 weeks, or even longer. Consequently to appraise adequately the significance of a large number of dead, one must consider the bird population that has previously frequented the area.

REMEDIAL MEASURES

Having learned the cause of a malady, the inquiry naturally follows: What can be done about it? In the case of duck sickness the efficacy of certain remedial measures had been demonstrated long before the actual cause had been ascertained. Though the reasons for the relief attained were misunderstood, there was ample demonstration of the usefulness of the methods then employed.

Remedial measures include both direct treatment and prevention. Under the former will be discussed the utility of antitoxin and other therapeutics as well as the benefits to be derived from rescue work and the so-called "fresh water" treatment. Under prevention will be considered procedures involving (1) deep flooding or drying of sickness areas; (2) employment of frightening measures to drive birds out of known infected areas; and (3) decrease of infection and toxin production by sanitation, including proper carcass disposal.

TREATMENT OF BIRDS

ANTITOXIN

An antitoxin against type C botulism has been developed by methods ordinarily employed in the production of antiserums. Goats or horses that have been immunized by the administration of toxin in small doses over long periods are the usual sources of antitoxins.

Injected in proper doses, simultaneously with or soon after the ingestion of toxin, such antitoxins protect the bird or other animals treated.

Little if anything, however, of practical value can be expected from the use of antitoxin as a combative measure against any malady of wild ducks. Although research workers in the field of botulism have shown that antitoxin administered to experimental animals even after symptoms have developed possesses some slight therapeutic value (*3, p. 83*), such animals cannot be saved if the antitoxin treatment is delayed until the symptoms are well advanced (*6*). A wild bird so incapacitated by duck sickness as to permit its capture for treatment would have ingested the toxin hours or even days previously, and hence would be at a disadvantage in an attempt to cure by means of antitoxin.

That several contacts with duck sickness fail to set up an immunity in wild ducks also argues against the possibility of ever developing any practical prophylactic treatment with antitoxin. It could hardly be expected that artificially induced immunity would endure over long periods or to a degree that would cope with the superlethal doses often ingested.

A pronounced tolerance for the toxin of *Clostridium botulinum*, however, may be encountered now and then in individual wild birds used for experimental purposes. A case in point, of which there were several in the course of these studies, was that of a pintail, which had assimilated two successive oral doses of 0.2 g of toxic material without manifesting symptoms, whereas a 0.1-g dose ordinarily produced a typical case of duck sickness.

RESCUE OF AFFECTED BIRDS

Next to such preventive measures as elimination of extensive infected areas or driving the birds from them, the rescue and subsequent care of affected birds probably has yielded the greatest returns in the battle against duck sickness. A high percentage of the birds rescued (pl. 6, *B*) and brought into pens where they can be given direct attention, recover. Experimental evidence, however, is still insufficient to indicate the extent that birds recover under natural conditions; and, therefore, despite the gratifying results attained in rescue operations, the real value of such work is uncertain. The mortality is the result of the combined effects of the toxin of botulism and various other attendant factors to which the helpless birds are exposed in nature, including the devastating heat of mid-day, attacks of predators, feather-soaking, chilling at night, and even drowning. When these dangers are minimized or eliminated, as is the case with rescued birds, a greater degree of recovery can be expected, and it is on this basis that rescue operations may be advocated.

In the course of his work at Great Salt Lake, Wetmore (*71*) kept records of the results attained in rescue operations, involving 1,211 ducks of 7 species. Of these, 284 died and 927 recovered. After making allowance for a large number of very sick birds, which, except for experimental purposes, would not have been brought in for treatment, Wetmore concluded that the ratio of recovery would be about 90 percent of the birds rescued. The results, when tabulated (including all birds rescued), indicated that of the three species most

often affected, the green-winged teal showed the lowest degree of recovery (75 percent), the pintail next (77 percent), and the mallard the greatest (81 percent).

The percentage of recovered birds was somewhat lower at Tule Lake than at Great Salt Lake. A number of factors accounted for this. In the first place, rescue work at Tule Lake was for experimental purposes and not with the primary objective of salvaging birds. In addition, the birds had to be transported 30 to 45 miles by automobile to the experimental pens. Even with the greatest of care many died during these trips.

Details of rescue methods often have to be worked out to meet local conditions. At Great Salt Lake, where travel on some of the extensive mud flats is extremely arduous, transportation problems have been met in part by the use of narrow, flat-bottomed boats of shallow draft, having paddle wheels driven by a gasoline engine. This peculiar but efficient type of boat, passing under the local name of "mud queen", can be operated in very shallow water and used to tow one or more even shallower-draft boats for transporting the sick ducks. At Tule Lake conditions for capturing sick birds were more favorable, since, with the exception of a few areas, the bottom was fairly firm and in 1929 and 1930 the water depth was such that nearly the whole lake could be traversed on foot. In those seasons a rubber boat or light skiff, pulled along by hand, was used to gather the sick birds.

At Lake Malheur, however, in 1929 the difficulties of rescue work were greater. Water levels were so low that navigation in any kind of boat was slow and arduous and progress on foot was prevented by the almost fathomless mud. Under such conditions rescue work could cover nothing more than the main channels of deeper water. At other points, as in some areas adjacent to the Salton Sea and on the grounds of certain duck clubs elsewhere in California, best progress in rescue work was made simply by searching the borders of levee banks, where wind and wave action had drifted the helpless birds. These could be gathered and placed in a light skiff pulled along in the deeper water of the adjacent borrow pit.

In gathering sick ducks on open-lake or mud-flat areas two or three men can work to advantage in loading a single boat. When the birds are widely scattered and the flats can be traversed on foot the men can best bring small lots to the boat by placing the birds in loosely woven burlap or netting sacks, or in baskets. When they are transferred to the boat, or "duck ambulance", the larger species should be separated from the smaller, and the vigorous from those that are prostrate.

The question often arises as to whether one should endeavor to rescue all sick birds encountered, regardless of their degree of disability. The answer depends upon the facilities for transportation and subsequent care. If conditions affecting rescue work are favorable throughout, no bird found alive need be overlooked. Under conditions adverse for handling, birds that are utterly prostrate may as well be left and efforts confined to those less seriously affected. Birds still active should not be passed by on the assumption that they are not seriously affected or are on the road to recovery, as exactly the opposite may be the case.

When there are many rescued birds so active that they can readily scramble out of an ordinary boat, their escape can be prevented by draping a fish seine or other netting over the top and fastening it at intervals. A few rushes or similar vegetation strewn lightly over the birds will afford protection from the direct rays of the sun and instill in the more restless ones a sense of security, thus reducing their desire to escape. Frequently a piece of light burlap thrown over particularly active birds will induce them to remain perfectly quiet. In transporting shore birds, particularly sandpipers, phalaropes, and other smaller species, pasteboard cartons are useful. These may be set in the bottom of the boat and the cover loosely closed to prevent the escape of active individuals.

Success in rescue work depends on the care given the birds at the recovery pens. A number of pens should be provided so that birds varying greatly in size or in degree of disability may be kept separate. Under some conditions it is an advantage to have portable cages. For small-scale operations, involving the salvage of a few hundred, it is convenient to have the floor size no greater than 6 by 12 feet, and the height 4 or 5 feet; the frame well constructed but light, and covered on the sides and top with 2-inch-mesh poultry wire. A 12-inch baseboard, forming the lower part of the frame, will reduce the danger of ducks injuring themselves by trying to escape through the wire. When it is planned to rescue thousands of birds, as has been done at Great Salt Lake, cages of larger dimensions, especially for the less severely affected or the nearly recovered birds, are more economical. One or more of these cages (depending on the number of birds being salvaged) should be so placed that part of the cage will stand in water or at least that an adequate water supply will be constantly available. In placing cages, stagnant water should be scrupulously avoided. Other cages, in which water is supplied in small containers into which the birds cannot fall, should be provided for those more severely affected but still able to move about. For birds that are utterly prostrate or for those unable or not inclined to eat or drink, perfectly dry cages, well bedded with a supply of straw or dry grass, should be provided. All cages should have a certain protection from the rays of the midday sun, particularly those for prostrate birds.

Small cages for the care of the most severely affected birds may be placed to advantage on racks or tables raised about 4 feet from the ground. A single box cage of this type was used at the recovery pens at Klamath Falls. Birds kept in it could be given drinking water through a pipette or medicine dropper and otherwise readily attended to. A series of such "receiving wards", each large enough to hold 15 or 20 birds without crowding, will permit attention in an orderly manner and lessen the danger that extremely weak birds may become buried under others floundering about.

The methods outlined are in the nature of ideals to be sought wherever possible, though under the pressure of strenuous field operations they may not always be feasible. Outbreaks of duck sickness come quickly and often disappear in the same manner. There is no way to foretell their extent or severity, and birds usually are salvaged as best they can be with the facilities at hand. Let

it not be forgotten, however, that a duck rescued is equivalent to a duck raised. Madsen (*45, p. 109*) has estimated that, under conditions prevailing at Great Salt Lake, ducks may be reclaimed at a cost of 5 to 10 cents apiece, which is certainly trivial compared with the cost of game-bird propagation. In former severe outbreaks it was estimated that, had facilities been suitable and funds ample, 25,000 to 50,000 birds could have been salvaged on the Bear River Marshes alone.

Since the desire to feed is the last of the impaired major impulses to be regained by birds recovering from duck sickness, the question of food does not enter into the care of sick birds until they have made substantial progress toward recovery. The period during which they need to be fed is not long—usually not more than 4 to 6 days and, in the case of individuals captured while recuperating, the period of detention need be only a day or two. Ordinarily nothing practical can be done in the feeding of the highly insectivorous shore birds, and for this reason they should be released as soon as they regain their feet.

At Klamath Falls rolled barley was a satisfactory and readily obtainable food for convalescing birds of most species, including such diverse forms as teals, coots, and Canada geese. The barley ration was supplemented, however, every few days with green feed in the form of water cress (*Sisymbrium*) or duck weed (*Lemna*). The latter, as collected, contained many snails and insects, thus furnishing animal food as well.

When the idea prevailed that duck sickness was caused by the toxicity of certain soluble salts in alkali, the handling of affected birds in the manner described was referred to as the "fresh-water" treatment. It was inferred that fresh water, or at least the absence of more alkaline water, had effected the cure. With botulism determined as the cause, however, it will be well to reappraise the significance and merits of such treatment. In the first place there are to be borne in mind the circumstances surrounding the capture and subsequent treatment of birds that have been rescued, and the part these play in the high percentage of recoveries generally noted.

Ordinarily when sick birds are being gathered, those utterly prostrate are passed by, as also are the dead bodies that litter the mud flats by the thousands. The birds collected are either (1) those that are in the earlier stages of the onset, (2) those in process of recovery, or (3) those that have ingested sublethal doses of toxin. All the birds in the last two groups, and part of those in the first, have excellent chances for recovery, and any conclusions drawn from results attained with those and those only will be biased accordingly. The material on which such deductions are based is in fact hand picked and in no way reflects the true reaction of the disease. Were all the dead and nearly dead found in the field included, the percentage of recovery would be materially reduced.

It is to be remembered also that when birds are subjected to fresh-water treatment they usually are favored by several other factors, which though of the utmost importance to their recovery, have no connection with fresh water. Birds placed in a dry pen, sheltered from the heat of midday and the cold of night, safe from the

attacks of predatory creatures, the dangers of feather soaking and even drowning, often the fate of sick birds under field conditions, have advantages of far greater importance than any that might be derived from the mere oral administration of fresh water. Although there is reason to believe that fresh water, forcibly and carefully given to helpless birds, will aid somewhat in the restoration of normal physiological processes, there is no evidence that it has any direct curative or combative value in cases of botulism.

If the fresh-water treatment should consist merely of giving the affected birds access to water, of which they are to avail themselves as best they can, the results will be fatal in a large proportion of the cases, owing to feather soaking, chilling, or drowning. If attention cannot be given individual birds and if those in varied stages of the disease cannot be properly segregated, a policy of no water or of water only in proper containers is preferable to an open supply into which the helpless birds may flounder.

PREVENTION

WATER HANDLING

From the very nature of duck sickness the most effective measures of relief are those of prevention rather than cure. The malady is definitely associated with areas of shallow water or mud flats. Whereas formerly these mud flats with their thin film of water or innumerable pools, evaporating sometimes to dryness, were looked upon as the source of the toxic salts considered to be the cause of the malady, they now become an important link in the chain of etiological factors that lead to the production of the toxin of botulism (p. 62), and its subsequent ingestion by birds. Any break in the sequence of these essential factors or events will effectively prevent or terminate an outbreak of duck sickness.

In South Africa, where lamsiekte (botulism of types C and D) is contracted by cattle that, by reason of a phosphate deficiency, possess a depraved appetite for bones, carrion, or other toxin-producing media, the situation is met by rectifying the phosphate deficiency in the diet or else by removing all carrion from pasture lands. When this is done lamsiekte is curbed, not through elimination of the organism, which may still be abundantly present, but by severing one link in the chain of events through which cattle obtain doses of toxin. Were it possible to eliminate, with equal thoroughness, all toxin-producing media from infected areas, duck sickness also could be effectively controlled. With a host of diverse media present, however, and likely to be ingested by waterfowl, shore birds, or other species, the possibility of accomplishing this through any labored process of detection and removal is wholly out of the question.

It is possible, however, to eliminate mud flats and shallow-water areas, either by flooding sufficiently to submerge them or by evaporation to the point of dryness. By the first of these processes high temperatures, favorable to toxin production, are reduced, and through dilution and water movement, any toxin that may have been formed is dispersed or attenuated to the point of harmlessness.

Furthermore, replacing mud flats with deep water often makes infected areas less attractive as loitering or feeding grounds for waterfowl and shore birds. Even more effective severance of the etiological chain may be accomplished by complete dessication of the infected areas. The simple expedient of cutting off the water supply and allowing the flats to dry was used in combating duck sickness in the marshes at the mouth of the Jordan River, Utah, as early as 1911.

With the establishment and subsequent development of the Bear River Migratory Bird Refuge, steps were taken to meet the ever-recurring menace of duck disease. Rapid and effective handling of water levels was impossible through drainage alone, owing to the great expanse and the level character of the delta on which the refuge rests. It was found necessary not only to raise a barrier against an influx of saline water from Great Salt Lake itself but to subdivide the inclosed area by means of other dikes in such manner as to permit either deep flooding with fresh water coming from Bear River or complete drying of the various units and smaller sections of the refuge.

On April 3, 1928, in the act establishing Bear River Migratory Bird Refuge, Congress authorized an appropriation of $350,000 to carry on such a program. Work was begun in 1929, and by July 1930 the dikes surrounding 3 of the 5 units comprising the refuge had been practically completed. The engineering work of the whole project was finished during the next year. This included a great outer dike approximately 20 miles long, which in seasons of plentiful water supply can impound about 25,000 acres of water. By means of gates and drainage spillways as many of the units may be flooded as the seasonal water supply permits.

At clubs or on private estates in infected areas where artificial ponds are formed by the diking and flooding of lands, precautions must be taken to avoid conditions conducive to toxin production. Water levels should be reasonably deep (1 or more feet near shore and greater in the center). Water temperatures should be kept below 80° to 90° F. during midday by circulation or change of the water or by increasing its depth. Shore lines should be kept well defined and clean. Feeding should be either on sand or gravel beaches prepared for the purpose or in troughs away from the water; promiscuous broadcasting of grain in shallow water or on mud flats should be avoided.

FRIGHTENING MEASURES

The employment of frightening measures to drive waterfowl out of known infected areas has been resorted to with marked beneficial results on the Bear River flats. As early as 1921 officials of the game department of Utah adopted the practice of patrolling the flats, beginning early in August of each year (*45*). At the first signs of duck sickness, high-powered rifles were used to keep the flocks of healthy birds away from these areas. This was continued until fall rains, a rise in water level, or the lowered temperatures of October ended the possibility of an outbreak. The practice of rifle patrol, instituted and carried out by State officials from 1921 to 1928, was continued by Federal officials even after the establish-

ment of the Bear River Migratory Bird Refuge. In the short-lived but severe outbreak of August 1929 it was considered that gunfire, which drove flocks of healthy birds away from an infected area at the mouth of the Bear River, did much towards preventing an even greater epizootic.

SANITATION

Disposal of the bodies of dead birds found in duck-sickness areas was one of the earliest of the measures used against the disease in Utah (p. 33). The procedure appeared to be logical and it probably did some good, even though the futility was soon impressed on the small crew of workers then participating in the work. Madsen's description (*45*) of these early operations is quoted on page 33.

The present study has definitely established the fact that the bodies of birds dying of duck sickness are excellent media for the production of toxin and may become the direct source of infection for other birds (p. 59). Sarcophagid larvae, which have developed in these bodies, also are sources of toxin and are likely to be picked up by shore birds as well as ducks. There is no question that the removal and proper disposal of dead bodies reduces in the aggregate the toxin available to birds, but evidence still is lacking as to the degree of improvement attained thereby. In view of the fact that a host of other toxin-producing media, including the remains of insects and various other invertebrates, as well as grain and decaying vegetation, may be contributing to the general infection, even energetic measures of body disposal may result in only a slight improvement. Until more is learned by field experimentation of the role of dead bodies in a duck-sickness outbreak, it would seem that efforts might better be spent in frightening healthy birds away from body-strewn areas, and in the rescue and subsequent cure of afflicted birds. The case is quite different from that of lamsiekte in South Africa, where the clean-up of carrion in infected pastures has brought beneficial results. There the sources of toxin are relatively few and discernible; in duck sickness, the toxin may come from a multitude of media, some of them quite obscure.

Treatment of infected areas with antiseptics or other substances, with the object of destroying the causative organism, preventing toxin production, or neutralizing toxin already formed, is a matter to which some consideration has been given though no field experimental work on it has been done. The immensity of the infected areas and the difficulty of reaching submerged spots of toxin, as well as the deleterious effect of powerful antiseptics on vegetation and possibly even on wild fowl, are obstacles that at this time make such treatment impracticable.

Whether a chemical treatment of infected areas so as to change them from a condition of alkalinity to one of neutrality or even acidity with respect to the hydrogen-ion concentration, would improve matters is yet to be determined. It is a fact that the range of duck sickness as it occurs in epizootic form coincides, to a marked degree, with that of the alkaline waters of this country. It is true also that the toxin of botulism is elaborated to a greater degree or in more potent form in alkaline media. The prospect of attacking the duck-sickness problem, however, through altering on an extensive

scale the hydrogen-ion concentration in waters frequented by the birds does not appear promising, though the procedure may be worth trial in localized spots of infection.

SUMMARY

Earlier studies of disastrous outbreaks of what has been termed "western duck sickness", summarized in bulletins issued by the Department of Agriculture in 1915 and 1918 (*70, 71*), pointed to the cause as "the toxic action of certain soluble salts found in alkali." Later evidence that this explanation was not adequate led the Bureau of Biological Survey to resume its studies in 1927, and by 1931 the results attained had removed the problem from the field of chemical toxicology to that of bacteriology. The malady is now recognized as a form of botulism, a disease caused by the toxin produced by a common saprophytic and anaerobic bacterium, *Clostridium botulinum*, type C.

This organism, thriving under conditions of decay, had previously been recognized as the cause of more or less localized outbreaks of limberneck among domestic poultry and forage poisoning in livestock. Its association with the extensive wild-fowl epizootics has not only altered earlier concepts of this ailment of wild birds, but it has changed, even more radically, the bacteriologist's idea of the prevalence, history, and importance of *Clostridium botulinum*, type C.

In distinction from many contagious bacterial diseases, outbreaks of botulism, which is simply a form of food poisoning, are not dependent on a weakened or predisposing condition of the victim. Factors controlling the prevalence of the disease are largely those that affect the welfare of the organism itself and the production of its toxin. Among these are the prevalence of dead organic matter, animal or vegetable; an alkaline environment, which plays an important part in the bacterial processes of toxin production; a condition of shallow water or stagnation, preventing toxin dilution or dispersal; and relatively high temperatures.

Diagnosis of botulism as the cause of duck sickness has been based in this study on (1) the similarity of the clinical picture of the disease in the field with that produced experimentally by the administration of the toxin of pure cultures of type C *botulinum;* (2) the frequent recovery of the causative organism from the tissues of affected birds in distinction from its absence from the tissues of healthy birds; (3) the demonstration in the field of the toxin of type C *botulinum* in foods and water commonly ingested by birds; and (4) on the fact that the incidence, course, and disappearance of duck sickness in the field conforms to the influence of environmental factors in a manner highly suggestive of botulism.

This study has shown that such bird-food items as sarcophagid-fly larvae and miscellaneous insect debris (acceptable to shore birds and ducks), submerged grain (eagerly sought by ducks), and the bodies of birds dying of the disease (a common food item of gulls) are some of the channels through which the poison may be taken. Toxin also has been demonstrated in the mud of infected areas rich in organic matter, and even the water of small pools may harbor the poison produced in nearby media.

The toll from the disease, though varying greatly from year to year, must be looked upon as the greatest drain upon western waterfowl due to any single natural agency. An estimated loss of a quarter of a million birds at the north end of Great Salt Lake in the summer and fall of 1932 points to the current importance of the malady as a destroyer of wild life. Not only ducks but a multitude of other birds succumb: at present the list of known victims comprises 69 species, in 21 families of wild birds. Although various vertebrates other than birds are susceptible to the toxin of type C *botulinum*, evidence points to the fact that human beings are relatively, if not absolutely, immune to oral doses.

The history of western duck sickness has been traced, with a fair degree of accuracy, back to the early nineties of the last century. The year 1910 marked the point when Nation-wide attention was first called to the malady as a result of the publicity given the disastrous outbreak in the marshes about Great Salt Lake in that year. Since that time the disease has occurred at an ever-increasing number of localities in the West, and today the distribution of the disease among North American wild fowl conforms in general to the region of alkaline waters and soils of the Western States and the southern Canadian Provinces. Although the causative organism was probably endemic in North America long before it was evidenced in outbreaks of duck sickness, it is apparent that conditions conducive to epizootics have been accentuated by man through his utilization of water supplies. This often has resulted in the creation, during periods of hot weather, of alkaline sinks and areas of shallow, stagnant water, mud flats, and their associated decaying organic matter, in which toxin may be produced.

Remedial measures may be in the form of direct treatment or prevention. Among the former are the rescue and subsequent care of afflicted birds. These will save many that would otherwise succumb to such attendant hazards as the heat of midday, attacks of predators, feather soaking and chilling at night, and even drowning. The use of antitoxins under field conditions is impractical. At points of local infection much sickness may be prevented by frightening the birds, through rifle fire, from dangerous ground.

More lasting and effective means of prevention, however, lie in modifying or eliminating conditions that favor the development of the organism and the evolvement of its toxin. By flooding mud flats and shallow, stagnant water areas with deep or flowing water, temperatures are reduced and, through a process of dilution, preformed toxin may be attenuated to the point of harmlessness. The expedient of temporarily cutting off all water from infected areas also has been used effectively where the ground may be completely desiccated and thus become unattractive to birds in midsummer.

An inadequate water supply and fluctuating water levels are the dominant environmental factors conducive to outbreaks of duck sickness. Often these are the direct result of man's increasing diversion of the supply from favorite wild-fowl areas, where formerly reasonable and more nearly constant water depths were maintained throughout periods of hot weather. Unless these conditions can be restored, duck sickness will continue to take, even increasingly, its annual devastating toll of western wild fowl.

LITERATURE CITED

(1) AUSTIN, O. L., and AUSTIN, O. L., JR.
1931. FOOD POISONING IN SHORE BIRDS. Auk 48: 195–197.

(2) BENGTSON, I. A.
1923. A TOXIN-PRODUCING ANAEROBE ISOLATED PRINCIPALLY FROM FLY LARVAE. ITS RELATION TO THE ORGANISMS HITHERTO KNOWN TO BE CAUSATIVE FACTORS IN THE PRODUCTION OF BOTULISM. Pub. Health Rpts. [U.S.] 38: 340–344.

(3) ———
1924. STUDIES ON ORGANISMS CONCERNED AS CAUSATIVE FACTORS IN BOTULISM. U.S.Pub. Health Serv., Hyg. Lab. Bul. 136, 101 p., illus.

(4) BIDAULT, C.
1924. INFLUENCE DE CERTAINS CATIONS SUR LA CULTURE EN BOUILLON DE BACILLUS BOTULINUS. Compt. Rend. Soc. Biol. [Paris] 90: 1135–1136.

(5) BURKE, G. S.
1919. NOTES ON BACILLUS BOTULINUS. Jour. Bact. 4: 555–570, illus.

(6) BURKE, V., ELDER, J. S., and PISCHEL, D.
1921. TREATMENT IN BOTULISM. Arch. Int. Med. 27: [265]–304.

(7) [CALIFORNIA] FISH AND GAME COMMISSION.
1913. WILD DUCK EPIDEMIC AT TULARE LAKE. Amer. Field 80: 350.

(8) CLARKE, F. C.
1913. PRELIMINARY REPORT UPON THE DISEASE OCCURRING AMONG THE DUCKS OF THE SOUTHERN SAN JOAQUIN VALLEY DURING THE FALL OF 1913. Condor 15: 214–226, illus.

(9) CLARKE, F. W.
1924. THE DATA OF GEOCHEMISTRY. U.S.Geol. Survey Bul. 770, ed. 5, 841 p.

(10) COLEMAN, G. A.
1929. A BIOLOGICAL SURVEY OF SALTON SEA. Calif. Fish and Game 15: 218–227.

(11) COWDRY, E. V., and NICHOLSON, F. M.
1924. AN HISTOLOGICAL STUDY OF THE CENTRAL NERVOUS SYSTEM IN EXPERIMENTAL BOTULINUS POISONING. Jour. Expt. Med. 39: 827–836.

(12) DERBY, G. H.
1852. REPORT OF THE SECRETARY OF WAR COMMUNICATING . . . A REPORT OF THE TULARE VALLEY. (U.S.Engin.Dept.) 17 p., illus. [U.S.] Cong. 32d, 1st sess., Senate Ex. Doc. 110.

(13) DICKSON, E. C.
1915. BOTULISM, AN EXPERIMENTAL STUDY. A PRELIMINARY REPORT. Jour. Amer. Med. Assoc. 65: 492–496, illus.

(14) ———
1918. BOTULISM. A CLINICAL AND EXPERIMENTAL STUDY. Rockefeller Inst. Med. Research Monog. 8, 117 p., illus.

(15) ———
1918. BOTULISM; A FURTHER REPORT OF CASES OCCURRING IN THE PACIFIC COAST STATES. Arch. Int. Med. 22: [483]–495.

(16) ———
1926. THE PRESENT STATUS OF THE BOTULISM PROBLEM. X. PATHOGENICITY, TOXICOLOGY, AND IMMUNOLOGY. Jour. Prev. Med. 1: 71–83.

(17) DOZIER, C. C.
1924. INHIBITIVE INFLUENCE OF SUGARS AND SALT ON VIABILITY, GROWTH, AND TOXIN PRODUCTION OF B. BOTULINUS. XVII. Jour. Infect. Diseases 35: [134]–155, illus.

(18) EDMUNDS, C. W., and LONG, P. H.
1923. CONTRIBUTION TO THE PATHOLOGIC PHYSIOLOGY OF BOTULISM. Jour. Amer. Med. Assoc. 81: 542–547.

(19) ERMENGEM, E. VAN
1897. CONTRIBUTION À L'ÉTUDE DES INTOXICATIONS ALIMENTAIRES: RECHERCHES SUR DES ACCIDENTS À CARACTÈRES BOTULINQUES PROVOQUÉES PAR DU JAMBON. Arch. Internatl. Pharmacodyn. et Ther. 3: 213–350, 499–601, illus.

(20) ERMENGEM, E. VAN
1897. UEBER EINEN NEUEN ANAËROBEN BACILLUS UND SEINE BEZIEHUNGEN ZUM BOTULISMUS. Ztschr. Hyg. u. Infektionskrank. 26: 1–56, illus.

(21) FISHER, A. K.
1893. REPORT ON BIRDS [Death Valley expedition]. U.S.Dept.Agr., Bur. Biol. Survey, North Amer. Fauna no. 7: 7–158.

(22) FRÉMONT, J. C.
1845. NARRATIVE OF THE EXPLORING EXPEDITION TO THE ROCKY MOUNTAINS IN THE YEAR 1842 AND TO OREGON AND NORTH CALIFORNIA IN THE YEARS 1843–'44. 278 p. ([U.S.] Cong. 28th, 2d sess., Senate Ex. Doc. 174.)

(23) GAHRINGER, J. E.
1926. THE SENSITIZATION OF PIGEONS TO FOREIGN PROTEINS. Jour. Immunol. 12: 477–488.

(24) GEIGER, J. C., DICKSON, E. C., and MEYER, K. F.
1922. THE EPIDEMIOLOGY OF BOTULISM. U.S.Pub. Health Serv. Bul. 127, 119 p., illus.

(25) GILTNER, L. T., and COUCH, J. F.
1930. WESTERN DUCK SICKNESS AND BOTULISM. Science (n.s.) 72: 660.

(26) GRAHAM, R., and BOUGHTON, I. B.
1923. CLOSTRIDIUM BOTULINUM, TYPE C. A PATHOGENIC ANAEROBE ASSOCIATED WITH A LIMBERNECK-LIKE DISEASE IN CHICKENS AND DUCKS. Ill. Agr. Expt. Sta. Bul. 246, 34 p., illus.

(27) GUNDERSON, M. F.
1932. THE OCCURRENCE OF LEAD POISONING AND WESTERN DUCK SICKNESS IN MINNESOTA. Fins, Feathers, and Fur 106: 8–9, 23.

(28) ———
1933. PRESENCE OF CLOSTRIDIUM BOTULINUM IN LIVERS OF BIRDS NOT AFFECTED WITH BOTULISM. Soc. Expt. Biol. and Med. Proc. 30: 747–750.

(29) GUNNISON, J. B., and COLEMAN, G. E.
1932. CLOSTRIDIUM BOTULINUM, TYPE C, ASSOCIATED WITH WESTERN DUCK DISEASE. Jour. Infect. Diseases 51: 542–551.

(30) ——— and MEYER, K. F.
1928. SUSCEPTIBILITY OF MACACUS RHESUS MONKEYS TO BOTULINUM TOXIN TYPE B, C, AND D. Soc. Expt. Biol. and Med. Proc. 26: 89–90.

(31) ——— and MEYER, K. F.
1929. CULTURAL STUDY OF AN INTERNATIONAL COLLECTION OF CLOSTRIDIUM BOTULINUM AND PARABOTULINUM. XXXVIII. Jour. Infect. Diseases 45: [119]–134, illus.

(32) ——— and MEYER, K. F.
1930. SUSCEPTIBILITY OF MONKEYS, GOATS, AND SMALL ANIMALS TO ORAL ADMINISTRATION OF BOTULINUS TOXIN, TYPES B, C, AND D. Jour. Infect. Diseases 46: [335]–340.

(33) HART, G. H.
1920. BOTULISM IN CHICKENS. Jour. Amer. Vet. Med. Assoc. (n.s. 10) 57: 75–77.

(34) HELLER, V. G., and LARWOOD, C. H.
1930. SALINE DRINKING WATER. Science (n.s.) 71: 223–224.

(35) HENSHAW, H. W.
1875. REPORT UPON THE ORNITHOLOGICAL COLLECTIONS MADE IN PORTIONS OF NEVADA, UTAH, CALIFORNIA, COLORADO, NEW MEXICO, AND ARIZONA DURING THE YEARS 1871, 1872, 1873, AND 1874. *In* Wheeler, G. M., Report upon Geographical and Geological Explorations and Surveys West of the One Hundredth Meridian, v. 5, ch. 3, p. [131]–507, illus. Washington.

(36) HEWLETT, R. T., BULLOCK, W., and O'BRIEN, R. A.
1929. A SYSTEM OF BACTERIOLOGY (ch. 11, Bacillus botulinus). Medical Research Council, London.

(37) HILGARD, E. W.
1889. THE LAKES OF THE SAN JOAQUIN VALLEY. Calif. Agr. Expt. Sta. Bul. 82, 4 p.

(38) HOBMAIER, M.
1930. DUCK DISEASE CAUSED BY THE POISON OF THE BACILLUS BOTULINUS. Calif. Fish and Game 16: 285–286.

(39) HOBMAIER, M.
1930. DUCK DISEASE CAUSED BY THE TOXIN OF CL. BOTULINUM TYPE C. Soc. Expt. Biol. and Med. Proc. 28: 339–340.

(40) ———
1932. CONDITIONS AND CONTROL OF BOTULISM (DUCK DISEASE) IN WATERFOWL. Calif. Fish and Game 18: 5–21.

(41) KALMBACH, E. R.
1930. WESTERN DUCK SICKNESS PRODUCED EXPERIMENTALLY. Science (n.s.) 72: 658–659.

(42) ———
1932. PROGRESS IN WESTERN DUCK SICKNESS STUDIES. Science (n.s.) 75: 57–58.

(43) LOEW, O.
1876. REPORT OF THE ALKALINE LAKES, THERMAL SPRINGS, MINERAL SPRINGS, AND BRACKISH WATERS OF SOUTHERN CALIFORNIA AND ADJACENT COUNTRY. *In* Wheeler, G. M., Annual Report Geographical Surveys West of the One Hundredth Meridian in California, Nevada, Utah, Colorado, Wyoming, New Mexico, Arizona, and Montana. Appendix H3 of Appendix JJ.

(44) LUNDLUN, R. E.
[1928]. REPORT OF THE BUREAU OF RESEARCH. Calif. Dept. Nat. Resources, Fish and Game Comm. Bien. Rpt. (1926–28) 30: 74–78, illus.

(45), MADSEN, D. H.
1929. THE TRAGEDY OF WESTERN WATERFOWL. Field and Stream 33 (12): 30–31, 108–110, illus.

(46) MEYER, K. F., and DUBOVSKY, B. J.
1922. THE DISTRIBUTION OF THE SPORES OF B. BOTULINUS IN THE UNITED STATES. IV. Jour. Infect. Diseases 31: [550]–594, illus.

(47) ——— and DUBOVSKY, B. J.
1922. THE OCCURRENCE OF THE SPORES OF B. BOTULINUS IN BELGIUM, DENMARK, ENGLAND, THE NETHERLANDS, AND SWITZERLAND. VI. Jour. Infect. Diseases 31: [600]–609, illus.

(48) ———
1928. BOTULISMUS. *In* Kolle, W., and Wassermann, A. von, Handbuch der Pathogenen Mikroorganismen, Ed. 3, v. 4, lfg. 2, p. 1269–1304. Jena.

(49) MEYERS, J. T.
1925. RELATIONSHIP OF HARD WATER TO HEALTH. II. EFFECT OF HARD WATER ON GROWTH, APPEARANCE, AND GENERAL WELL-BEING. Jour. Infect. Diseases 37: 13–34, illus.

(50) MITCHELL, H. H., CARD, L. E., and CARMAN, G. G.
1926. THE TOXICITY OF SALT FOR CHICKENS. Ill. Agr. Expt. Sta. Bul. 279. p. 135–156, illus.

(51) MUNRO, J. A.
1927. THE WATERFOWL SICKNESS AT LAKE NEWELL, ALBERTA, 1925–1926. Canad. Field Nat. 41: 77–84.

(52) NELSON, E. W.
1927. VANISHING MIGRATORY BIRDS. Sat. Evening Post 199 (34): 12–13, 92, 94, 97, illus.

(53) O'ROKE, E. C.
1928. INTESTINAL PARASITES OF WILD DUCKS AND GEESE. Calif. Fish and Game 14: 286–296, illus.

(54) PALMER, C. C., and BAKER, H. R.
1922. BOTULISM (LIMBER NECK) IN SWANS. Ohio State Univ., Vet. Alumni Quart. 10: 93–94; illus.

(55) RIDGWAY, R.
1877. ORNITHOLOGY. *In* King, C., United States Geological Exploration of the Fortieth Parallel, v. 4, pt. 3, p. 303–669, Washington.

(56) ROBINSON, E. M.
1920. NOTES ON A FEW OUTBREAKS OF BOTULISM IN DOMESTICATED ANIMALS AND BIRDS. Union So. Africa Dept. Agr. Dir. Vet. Ed. and Research, Ann. Rpt. 15: 111–117.

(57) SCHÜBEL, K.
1923. ÜBER DAS BOTULISMUSTOXIN. Arch. Expt. Path. u. Pharmakol 96: [193]–259, illus.

(58) SEDDON, H. R.
1922. BULBAR PARALYSIS IN CATTLE DUE TO THE ACTION OF A TOXICOGENIC BACILLUS, WITH A DISCUSSION ON THE RELATIONSHIP OF THE CONDITION TO FORAGE POISONING (BOTULISM). Jour. Comp. Path. and Therapeutics 35: [147]–190, illus.

(59) SHAW, P. A.
1929. DUCK DISEASE STUDIES I: BLOOD ANALYSES IN DISEASED BIRDS. Soc. Expt. Biol. and Med. Proc. 27: 6–7.

(60) ———
1929. DUCK DISEASE STUDIES: II. FEEDING OF SINGLE AND MIXED SALTS. Soc. Expt. Biol. and Med. Proc. 27: 120–122.

(61) ———
1930. DUCK DISEASE STUDIES. III. SALT CONTENT OF SOILS IN DISEASE AND NONDISEASE AREAS. Soc. Expt. Biol. and Med. Proc. 27: 275–278, illus.

(62) ———
1930. RECENT PROGRESS IN DUCK DISEASE STUDIES. Jour. Amer. Vet. Med. Assoc. (n.s. 30) 77: 561–568.

(63) SHILLINGER, J. E.
1933. DISEASE AS A FACTOR IN WILD-LIFE ADMINISTRATION. Outdoor Amer. 11 (4): 10–12.

(64) STANSBURY, H.
1852. EXPLORATION AND SURVEY OF THE VALLEY OF THE GREAT SALT LAKE OF UTAH, INCLUDING A RECONNOISSANCE OF A NEW ROUTE THROUGH THE ROCKY MOUNTAINS. 487 p., illus. Philadelphia, ([U.S.] Cong. 32d, Spec. Sess., Senate Ex. Doc. 3.)

(65) THEILER, A.
1920. THE CAUSE AND PREVENTION OF LAMZIEKTE. Union So. Africa Dept. Agr. Jour. 1: [221]–244.

(66) ——— and others.
1927. LAMSIEKTE (PARABOTULISM) IN CATTLE IN SOUTH AFRICA. Union So. Africa Dept. Agr. Rpts. 11–12, Dir. Vet. Ed. and Research 2: 821–1361, illus.

(67) THOM, C., EDMONDSON, R. B., and GILTNER, L. T.
1919. BOTULISM FROM CANNED ASPARAGUS. Jour. Amer. Med. Assoc. 73: 907–912.

(68) TOPLEY, W. W. C., and WILSON, G. S.
1929. THE PRINCIPLES OF BACTERIOLOGY AND IMMUNITY. 2 v., illus. London.

(69) VAN ROEKEL, H.
1929. WILD DUCK DISEASES. Calif. Fish and Game 15: 11–12.

(70) WETMORE, A.
1915. MORTALITY AMONG WATERFOWL AROUND GREAT SALT LAKE, UTAH. (PRELIMINARY REPORT.) U.S. Dept. Agr. Bul. 217, 10 p., illus.

(71) ———
1918. THE DUCK SICKNESS IN UTAH. U.S. Dept. Agr. Bul. 672, 26 p., illus.

(72) ———
1919. LEAD POISONING IN WATERFOWL. U.S. Dept. Agr. Bul. 793, 12 p., illus. (Revised, 1922.)

(73) WYANT, Z. N., and NORMINGTON, R.
1920. THE INFLUENCE OF VARIOUS CHEMICAL AND PHYSICAL AGENCIES UPON BACILLUS BOTULINUS AND ITS SPORES. I. RESISTANCE TO SALT. Jour. Bact. 5: 553–557.

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