A PRELIMINARY STUDY OF THE SURVIVAL VALUE OF A FUNCTIONAL SALT GLAND IN PRAIRIE ANATIDAE

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THE presence in marine birds of the supraorbital, salt, or nasal gland has long been known to ornithologists but, until recently, its function apparently has been misconstrued. The occurrence, but not the function, of the gland in marine birds was known by Comelin in 1667 (see Technau, 1936), and its anatomy received considerable attention in the early nineteenth century (Nitzsch, 1820; Jacobson, 1813; Jobert, 1869). A review of early assumptions made concerning the function of the gland reveals a general thesis that the gland provided protection to the nasal mucosa by rinsing away excess salt (Marples, 1932; Schildmacher, 1932; Heinroth and Heinroth, 1926–1928; Technau, 1936). The correlation between marine habitat and size of gland also was noted previously, as was the ability of the gland to enlarge in response to exposure to increased salinity (Heinroth and Heinroth, 1926–1928; Schildmacher, 1932).

The excretory function of the gland was first correctly assessed by Schmidt-Nielsen, Jörgensen, and Osaki (1958). Since that time Schmidt-Nielsen and others stimulated by him have systematically investigated nearly every order and family of marine bird, including Diomedeidae (Frings *et al.*, 1958), Spheniscidae (Schmidt-Nielsen and Sladen, 1958), Alcidae and Hydrobatidae (Schmidt-Nielsen, 1960), Pelecanidae (Schmidt-Nielsen and Fänge, 1958c), Phalacrocoracidae (Schmidt-Nielsen *et al.*, 1958), and Laridae (Fänge, Schmidt-Nielsen, and Robinson, 1958).

The salt-excreting properties of the supraorbital gland in marine birds have recently been investigated extensively (Schmidt-Nielsen, 1959; Schmidt-Nielsen and Fänge, 1958a–c; Schmidt-Nielsen *et al.*, 1958; Fänge *et al.*, 1958; Scothorne, 1959a–c; Frings *et al.*, 1958; Frings and Frings, 1959; etc.). For detailed descriptions of the anatomy and physiology of avian salt glands see Schmidt-Nielsen *et al.* (1958), Schmidt-Nielsen (1960), and Scothorne (1959a, b).

To date all marine birds tested have been capable of ingesting and excreting within 12 hours sodium chloride in amounts equal to one-tenth of their normal body weights. It seemed to me that aquatic birds inhabiting the alkaline waters of western and central North America were subjected to physiological stresses similar to those confronting marine species. And, if marine birds had developed or maintained functional salt glands because of their environment, then it followed that waterfowl using prairie sloughs might well be similarly endowed.

Evidence resulting from research by Wetmore (1915) at Bear River,

Utah, that some prairie waterfowl possess functional salt glands was presented by Kalmbach and Gunderson (1934: 21). Wetmore had given "alkali" to waterfowl and had noted a nasal discharge which he attributed —erroneously it would now seem—to involvement of the Harderian gland. The later investigations of Scothorne (1959a–c) added proof that at least some Anatidae possess functional salt glands.

Further, Schmidt-Nielsen *et al.* (1958) and Schmidt-Nielsen (1959) had stated that anesthetics could not be administered to test birds because this caused the salt gland to cease functioning, and these statements provided most of the impetus to the present study. They suggested a possible link between "salt water intoxication," a functional supraorbital gland, and avian botulism.

The salt gland is innervated by a peripheral branch of the facial, or seventh, cranial nerve. The gland apparently responds to acetylcholine (Hokin and Hokin, 1959), released after stimulation of osmoreceptors. The neurotoxin produced by *Clostridium botulinum* Type C effectively inhibits the release of acetylcholine (Stover *et al.*, 1953). Yet, except in extreme cases, treatment of paralyzed birds with fresh water is equally as effective as antitoxin in overcoming the "disease." That effects of a neurotoxin could be ameliorated after neural involvement, by a drink of fresh water, did not at first appear to me to make sense. It would make sense, however, if the symptoms of avian botulism, or "duck disease," were in fact symptoms of osmoregulatory breakdown resulting from the inhibition of a functional salt gland.

This study, therefore, was begun in late 1959 to investigate the hypothesis that ducks, gulls, grebes, and shorebirds inhabiting the Great Plains of North America must possess a functional salt gland in order to cope with the highly alkaline and saline waters of many prairie sloughs and lakes; I hoped, further, to establish a relationship between the salt gland and avian botulism.

These objectives were to be accomplished, if possible, by (a) determining the presence or absence of a functional nasal gland in as many species as possible, (b) by studying the effect of various anesthetics on the ability of the gland to secrete salt, and (c) testing the effects of *Clostridium botulinum* Type C toxin on the functional ability of the gland.

Birds used in the study were supplied by the Delta Waterfowl Research Station, Delta, Manitoba, and the Bear River Research Station, Brigham City, Utah. Both wild and hand-reared individuals of the following species were studied: Mallards (*Anas platyrhynchos*), Pintails (*Anas acuta*), Green-winged Teal (*Anas carolinensis*), Blue-winged Teal (*Anas discors*), Shoveler (*Spatula clypeata*), Gadwalls (*Anas strepera*), American Widgeons (*Mareca americana*), Canvasbacks (*Aythya valisineria*), Redheads

TABLE 1

ANALYSIS OF THE EXCRETION OF THE SUPRAORBITAL GLANDS OF CERTAIN SPECIES OF WATER BIRDS, FOLLOWING ORAL ADMINISTRATION OF SODIUM CHLORIDE[1]

Species	*Nasal excretion*		
	Volume recovered[2] *(in cc)*	*Ions tested for (MEq/liter)*	
		Na^+	K^+
Mallard	13	605	21.7
Pintail	12	550	18.1
Ruddy Duck	12	570	18.9
Canvasback	+	–	–
Redhead	+	–	–
Green-winged Teal	+	–	–
Blue-winged Teal	+	–	–
Shoveler	+	–	–
Gadwall	11	590	20.3
American Widgeon	+	–	–
American Coot	+	–	–
Pied-billed Grebe	+	–	–
Killdeer	+	–	–
Franklin's Gull	+	–	–

[1] All birds were given 20 cc 10 per cent sodium chloride except the Pied-billed Grebe, Killdeer, and Franklin's Gull, which were given 5, 5, and 10 cc, respectively, of 10 per cent sodium chloride.

[2] Plus sign means that discharge was noted but was not available for measurements.

(*Aythya americana*) of wild stock only, Ruddy Ducks (*Oxyura jamaicensis*), American Coots (*Fulica americana*), Killdeers (*Charadrius vociferus*), Franklin's Gulls (*Larus pipixcan*), and Pied-billed Grebes (*Podilymbus podiceps*). The primary species used, however, were Mallards and Pintails. Groups of six birds of the same sex, age, and history were used in each experiment. Food and water were provided *ad libitum*.

THE EXPERIMENTS

1. *Determination of presence of functional salt gland.*—As seen in Table 1, every species tested possessed a functional salt gland.

The birds were tested individually for functional salt glands. Salt loads were given both intravenously (I.V.) into the brachial vein, and orally through a funnel attached to a catheter inserted as far as possible into the esophagus. Water was placed in the funnel and the catheter pulled back until flow began. Dissection revealed that the water load was placed in the proximal part of the proventriculus.

Throughout the test period, the birds were placed in a specially designed restraining box. The bill was taped down to prevent flicking of the head and subsequent loss of excreted material. A funnel was placed under the vent, and simultaneous collections were made of nasal and anal discharge. The volumes of the collections were noted at 30-minute intervals until flow from the nasal gland had ceased.

Collection of nasal and anal discharge was made directly into 10-ml graduated cylinders. Analyses for Na^+ and K^+ were made on a flame photometer supplied by the Department of Physiology, University of Manitoba Medical school. No analysis was made for the chloride ion, but all previous workers have demonstrated that its concentration is approximately equal to Na^+. Once I had established that a particular

TABLE 2
ANALYSIS OF ANAL AND NASAL EXCRETIONS OF AN UNANESTHETIZED MALE MALLARD FOLLOWING AN INTRAVENOUS INJECTION OF 15 CC OF 10 PER CENT SODIUM CHLORIDE

Elapsed time (minutes)	*Nasal excretion*			*Anal excretion*		
	MEq/liter of ions		*Volume (cc)*	*MEq/liter of ions*		*Volume (cc)*
	Na	*K*		*Na*	*K*	
30	588	26.2	2.0	–	–	trace
60	543	17.5	1.8	136.5	72.8	12
90	623	21.6	1.8	101.3	132.4	5
120	620	20.6	1.6	101.0	130.0	3
150	613	27.1	1.6	–	–	trace
180	643	18.6	1.7	–	–	trace
210	605	20.2	1.6	–	–	trace
Totals			12.1			20

species possessed a functional gland, additional experiments were conducted using free-ranging, unrestrained birds. These birds were caught periodically and any secreted material was placed on a glass slide. The results of evaporation on the slide indicated that the excretion contained a considerable amount of salt.

The initial tests were based upon the birds' responses to administration of 10 per cent sodium chloride. Subsequent testing with solutions of lower salinity (5 per cent and 2.5 per cent) indicated that these also produced a demonstrable nasal discharge.

The nature of the nasal excretion was similar to that described by Schmidt-Nielsen *et al.* (1958), and will not be discussed here. If the gland functioned at all, it followed the general pattern of excretion shown in Table 2.

It was noted that Pintails and Mallards supplied by the Bear River research station had glands which produced 40 per cent higher volume of nasal discharge than did those supplied by the Delta research station. (There was, however, no change from sample to sample in MEq/liter of Na^+ and K^+.) Also, the interval between "loading" and "peak response" was 30 minutes in the Delta birds but was extremely short in the Bear River birds. The increased volume and rapidity of response was probably a reflection of acclimation to salt concentration, which is greater in the water at Bear River Refuge, Utah, than at Delta Marsh, Manitoba. This point will be discussed in greater detail later.

We now pass to a consideration of experiments involving inhibition of salt gland function. Drugs and toxins used in these experiments were given intraperitoneally (I.P.) and orally, except epinephrine, which was administered intravenously.

2. *Effect of anesthetics.*—At the outset, I determined informally that a heavy but still subanesthetic dose (100 mg/kg body weight given I.P.) of Nembutal (pentobarbital sodium [Nembutal]) had a marked effect on volume of flow, MEq/liter of Na^+ and K^+ excreted, and survival. The results of a formal experiment with various doses of Nembutal (up to 50 mg/kg body weight, I.P.) are shown in Table 3. These experiments were conducted with birds under restraint. Since, however, I had also informally confirmed statements by Schmidt-Nielsen *et al.* (1958), that epinephrine would result in cessation of flow, and since it seemed possible that birds under restraint might be subject to a rise in endogenous epinephrine that would interfere with the ability of the gland to secrete, I conducted a duplicate experiment wherein birds similarly dosed with Nembutal were permitted to range freely. The results are shown in Table 4.

TABLE 3
EFFECTS OF VARYING DOSES OF PENTOBARBITAL SODIUM (NEMBUTAL) ON THE ABILITY OF RESTRAINED, WILD-CAUGHT MALLARDS TO EXCRETE SALT FOLLOWING A DOSE OF SODIUM CHLORIDE[1]

Nembutal dosage (mg/kg body weight)	*N*		*Mean volume of nasal excretion (cc)*	*MEq/liter of ions*			
				Nasal		*Anal*[2]	
	Tested	*Surviving*		Na^+	K^+	Na^+	K^+
None	6	6	13.1	604	21.7	118.9	102.6
10	4	3	12.1	600	22.1	115.0	102.0
20	5	3	10.1	580	20.0	–	–
30	4	2	9.1	430	15.5	trace	trace
50	4	0	7.0	373.8	7.1	trace	trace

[1] Salt load, for birds given no Nembutal and 50 mg/kg body weight of Nembutal, consisted of 15 cc of 10 per cent NaCl given intravenously. For all others the salt load was 30 cc of 10 per cent NaCl given orally.

[2] Dashes indicate that very little excretion occurred and analyses were not made.

Comparison of Tables 3 and 4 suggests the possibility that restraint may have contributed somewhat to mortality, although the difference seems scarcely conclusive. Unfortunately, no means of collecting measurable nasal excretion from free-ranging birds was devised and all that could be determined by continuous observation was the presence of a functional gland, the duration and (roughly) relative volume of flow, and survival.

3. *Effects of* Clostridium botulinum *toxin.*—The next phase of the investigation involved use of *Clostridium botulinum* Type C toxin. These experiments were conducted at Delta and at Bear River. Toxin used at Delta was supplied by Dr. V. C. Rowan-Walker, Connaught Laboratories, Toronto, and that used at Bear River was from a culture developed by Dr. Wayne I. Jensen, Bear River Research Station.

The initial experiments, at Delta, were conducted primarily to determine the amount of toxin (expressed in units of Mouse Lethal Doses = MLD) equal to the LD_{50} in various situations, as follows: (1) with the toxin administered either orally or I.P., and (2) in combination with either distilled water, 5 per cent NaCl, 10 per cent NaCl, or water from Louck's Pothole (Delta Marsh) which contained 50,000 ppm dissolved "salts."

LD_{50}'s, not used here in the conventional sense, mean "that dose killing 50 per cent of the birds within a given experiment." Because of variation in LD_{50} correlated with

TABLE 4
EFFECTS OF VARYING DOSES OF PENTOBARBITAL SODIUM (NEMBUTAL) ON THE ABILITY OF FREE-RANGING, WILD-CAUGHT MALLARDS TO EXCRETE SALT FOLLOWING AN ORAL DOSE OF 30 CC OF 10 PER CENT SODIUM CHLORIDE

Nembutal dosage (mg/kg body weight)	*N*		*Excretion*		*Duration of flow in number of 30-minute periods*
	Tested	*Surviving*	*Nasal*[1]	*Anal*	
None	6	6	++++	Yes	10
10	6	4	+++	Yes	8
20	5	3	++	Yes	6
30	5	3	++	Trace	2
50	6	1	+	Trace	1

[1] Volume of nasal discharge relative to that noted when no Nembutal was administered.

TABLE 5

LD_{50} OF *CLOSTRIDIUM BOTULINUM* TYPE C TOXIN FOR MALLARDS AND PINTAILS WHEN GIVEN ORALLY IN COMBINATION WITH ORAL LOADS OF 20 CC OF VARYING CONCENTRATIONS OF SODIUM CHLORIDE

Species	*N*	*Mean*[1] *and ranges*[2] *of* LD_{50}			*Pothole water*
		Fresh water	*10 per cent NaCl*	*5 per cent NaCl*	
Mallard	12	100,000 (50–150)	25,000 (20–30)	50,000 (40–60)	50,000 (35–65)
Pintail	12	75,000 (50–100)	20,000 (15–35)	35,000 (35–45)	35,000 (25–45)

[1] Expressed as mouse lethal doses.

[2] Expressed in thousands.

temperature changes, age, and physical condition of the test birds, results derived on one day could rarely be duplicated. Thus, LD_{50}'s given are averages of results obtained in three successive tests, each test being based on four birds. The range in LD_{50}'s obtained under varying conditions is given, in the tables concerned, in parentheses under each "average" LD_{50}.

The results of these initial experiments are given in Tables 5 and 6. The apparent differences between Mallards and Pintails may not be actual species differences, because of age and sex differences of the test animals; also, when converted to toxin units per unit of body weight, no real difference was found. As expected (probably because of such factors as the action of digestive enzymes and more variable absorption), oral doses produced extremely variable results, so all later experiments were conducted using I.P. administration. Toxin given intraperitoneally was always diluted with physiological saline to make all injections equal to one cc.

After average LD_{50}'s had been determined, the number of units administered was reduced gradually to the level where no demonstrable paralysis and very little, if any, weakening resulted. For both Pintails (see Table 7) and Mallards, that level was 250 units of the toxin given intraperitoneally when the birds were on fresh water. A series of birds first given 250 units of toxin was then given various salt and water loads as before.

Symptoms resulting from these treatments were arbitrarily divided into four classes of increasing severity: (1) weakness of legs, still capable of flying, nictitating membrane sluggish; (2) unable to move legs, nictitating membrane reflex absent, in-

TABLE 6

LD_{50} OF *CLOSTRIDIUM BOTULINUM* TYPE C TOXIN FOR MALLARDS AND PINTAILS WHEN GIVEN INTRAPERITONEALLY AND FOLLOWED BY ORAL LOADS OF 20 CC OF VARYING CONCENTRATIONS OF SODIUM CHLORIDE

Species	*N*	*Mean*[1] *and ranges*[2] *of* LD_{50}			*Pothole water*
		Fresh water	*10 per cent NaCl*	*5 per cent NaCl*	
Mallard	12	4,000 (35–45)	1,400 (12–16)	2,000 (18–22)	2,000 (15–25)
Pintail	12	3,000 (25–35)	1,000 (8–12)	1,500 (13–18)	1,500 (10–20)

[1] Expressed as mouse lethal doses.

[2] Expressed in hundreds.

TABLE 7
EFFECT OF VARYING DOSES OF *CLOSTRIDIUM BOTULINUM* TYPE C TOXIN GIVEN TO PINTAILS INTRAPERITONEALLY IN ASSOCIATION WITH 20 CC OF ORAL WATER LOADS OF VARYING SALINITY

Toxin[1] *dose*	*Water load*	*N*	*Mean time (in hours) to onset of symptoms,*[2] *and number of birds involved (in parentheses)*				
			Class 1	*Class 2*	*Class 3*	*Death*	*Per cent survival*
2,000	Fresh	6	27 (6)	42 (6)	48 (5)	60 (2)	66.6
1,500	"	6	36 (6)	42 (6)	61 (5)	66 (2)	66.6
1,000	"	6	40 (6)	80 (4)	–	–	100.0
500	"	6	48 (5)	72 (2)	–	–	100.0
250	"	6	54 (3)	–	–	–	100.0
2,000	5 % NaCl	6	12 (6)	17 (6)	22 (6)	30 (5)	16.6
1,500	"	6	17 (6)	21 (6)	27 (4)	48 (3)	50.0
1,000	"	6	21 (6)	27 (5)	45 (4)	58 (3)	50.0
500	"	6	27 (5)	36 (4)	48 (3)	62 (1)	83.4
250	"	6	33 (4)	40 (2)	–	–	100.0
2,000	10% NaCl	6	4 (6)	8 (6)	12 (6)	14 (6)	0.0
1,500	"	6	8 (6)	10 (6)	18 (6)	19 (4)	33.3
1,000	"	6	10 (6)	18 (5)	20 (4)	24 (3)	50.0
500	"	6	18 (5)	21 (4)	24 (3)	30 (2)	66.6
250	"	6	28 (5)	30 (4)	38 (2)	42 (1)	83.4
2,000	Pond	6	15 (6)	20 (6)	25 (6)	33 (5)	16.6
1,500	"	6	20 (6)	24 (6)	30 (4)	51 (3)	50.0
1,000	"	6	24 (6)	30 (5)	48 (4)	61 (3)	50.0
500	"	6	30 (5)	40 (4)	51 (3)	70 (1)	83.4
250	"	6	39 (4)	50 (2)	55 (1)	–	100.0

[1] In mouse lethal doses.

[2] See text for description of symptoms.

capable of sustained neck erection; (3) unable to lift head, complete general paralysis; (4) death.

Results for Pintails are given in Table 7. The lethal effect of even a 5 per cent salt load plus sublethal doses of toxin is readily apparent. Data in this table strongly support the thesis that effects of sublethal doses of toxin become lethal when combined with the effects of dissolved salts in ingested water.

An additional experiment (Table 8) consisted of giving a bird, in conjunction with given doses of toxin, two 20 cc salt loads of equal concentration, 12 hours apart. This experiment was an attempt to simulate a situation in the wild where a bird might take a second drink of water. A comparison of Tables 7 and 8 shows that in practically all instances, survival rates were lowered when a second salt load was given.

DISCUSSION

That the salt gland does have survival value has been demonstrated (Tables 3 and 4). Salt loads given to Mallards and Pintails produced an increased mortality rate associated with decreased salt gland activity, the latter being caused, in these experiments, by increasing dosages of Nembutal, which blocks parasympathetic control of the gland. The importance of the salt gland to survival arises when a bird has ingested a salt load greater than that which can be handled by the kidney. The gland serves

TABLE 8
SURVIVAL RATES OF PINTAILS GIVEN TWO 20 CC SALT LOADS OF EQUAL CONCENTRATION 12 HOURS APART, IN CONJUNCTION WITH GIVEN DOSES OF TOXIN GIVEN INTRAPERITONEALLY

Units toxin	*N*	*Per cent survival*			
		Fresh water	*10 per cent NaCl*	*5 per cent NaCl*	*Pond water*
2,000	10	100	00.0	00.0	00.0
1,000	10	100	00.0	30.0	40.0
500	10	100	50.0	60.0	70.0
250	10	100	70.0	90.0	90.0

as an accessory kidney, excreting only when the osmolarity of the plasma is elevated. It should be indicated here that, regardless of the cause of the increase in osmolarity of plasma (dextrose, sucrose, NaCl, etc.), discharge from the supraorbital gland consists mainly of sodium and chloride ions (Schmidt-Nielsen, 1960).

Heinroth and Heinroth (1926–1928) recognized that increased salt concentration in drinking water produced a remarkable increase in size and activity of the gland in Common Eiders (*Somateria mollissima*). Technau (1936) also noted that gulls had glands of varying size and activity in direct relation to their environment. The Great Black-backed Gull (*Larus marinus*) was most well adapted to a marine environment (largest salt gland), and the Mew Gull (*L. canus*) was least well adapted—a direct correlation between a purely marine species and a more inland species.

Analogous intraspecific variation occurs among Mallards and Pintails inhabiting the Great Plains of North America. In attempting to determine the presence, absence, or efficiency of a functional salt gland, I first tested wild adult males trapped at Delta Marsh. I found that some glands worked superbly, others moderately well, and still others, not at all. I consider this variation to be in part a reflection of immediate past history—some birds which were tested had just arrived from the acid waters of the taiga, with their low concentrations of dissolved solids, others were resident at Delta, and still others had emigrated from surrounding highly alkaline prairie areas. Once birds of wild stock reared at Delta were used, relatively uniform results were obtained. Later, Pintails reared at Delta were compared with those resident at Bear River, Utah, and I found that the latter group possessed glands which were 40 per cent more active than those from Delta—again, a reflection of the relative salinity of the habitat. Similar variation associated with habitat has been noted by Scothorne (1959a) in the domestic Mallard, and by Schmidt-Nielsen (1960) in various orders of sea birds. Such variation within a single species is of great significance. Especially important is the fact that in the absence of a salt load the gland

atrophies. It can and does respond to increasing salt loads after a time lapse of a duration as yet unknown.

How, though, does the existence of a functional salt gland in prairie anatids fit into the picture of avian botulism? There have been two main concepts of the etiology of what is now known as botulism. Wetmore's (1915) theory of "alkali poisoning" was discarded with the development of the bacterial intoxication theory of Gunnison and Coleman (1932) and Kalmbach and Gunderson (1934). More recently, Bell *et al.* (1955) have developed the microenvironmental concept (see discussion later) to suggest where and how birds could get lethal concentrations of toxin in the wild. Results of the present study strongly suggest that both theories noted above contain elements of truth, and, in fact, complement one another.

Birds may die of "botulism" in two ways: (a) from a massive dose of toxin, ingested while eating insect larval cases, or (b) from ingestion of sublethal doses of toxin, plus water or food containing relatively large amounts of dissolved salts. In the latter case, the functioning of the supraorbital gland appears to link the two causative agents. This is suggested by my experiments in which, in place of Nembutal, *Clostridium botulinum* Type C toxin was found apparently to block the parasympathetic nervous system or at least to interfere with the functioning of the salt gland.

Precise data on the efficiency of the salt gland following administration of toxin are unfortunately lacking. Facilities were not available at either the Delta or Bear River research stations for photometric analysis of nasal discharge. Observations made following toxin administration given in association with salt loads indicated that the gland was capable of excreting fluid at all levels of intoxication below the LD_{50}. Further, the amount of excretion increased as the toxin dose was reduced. The main effect was that the duration of flow was decreased in direct proportion to the dose of toxin. Extrapolation from the results obtained following administration of Nembutal suggests that the concentration of NaCl in the discharge decreases as the volume and duration of excretion is reduced. Thus, intoxication by *C. botulinum* toxin may have impaired the over-all effectiveness of the gland, both in terms of duration and quantity of flow and of concentrations of sodium, potassium, and chloride excreted. Associated with the decrease in volume and concentration of nasal discharge was an increase in total volume of fluid discharged via the kidneys.

I think, therefore, that death from a "sublethal" dose of the toxin plus a salt load (see Tables 7 and 8) is a result of the toxin's impairment of the effectiveness of the salt gland, and consequently the individual's incapacity to reduce the osmolarity of the blood plasma.

A brief résumé of the general conditions, and their timing, in North

America, under which botulism can be expected, will be helpful in understanding the problem.

Botulism is most prevalent in July, August, and September when air temperatures are high, water levels low, and concentrations of dissolved solids at a maximum. Botulism occurs along "feather edges" (areas of very shallow water where wind tides may push the water up over mud flats, accumulations of dead vegetation, and encrustations of "alkali"). During that period, birds may be moving from breeding areas to molting areas; they may travel among taiga, aspen parkland, and prairie. Finally, many birds may have been weakened by postnuptial molts, rigors of the breeding season, migration, or pathological phenomena.

The microenvironmental concept of botulism, put forth by Bell *et al.* (1955), is that *C. botulinum* can be incubated in the larvae or nymphs of *Phorma regina, Plectoptera* sp., etc.; the toxin is absorbed by birds when they eat the larvae. It is assumed that dead larval cases are freed from mud banks by wind tides and become a part of the "sludge-bed." There the carcasses are eaten, or the ambient fluid drunk. One earlier problem in confirming the Bell hypothesis has been that incubated larval carcasses yielded 10^4 MLD per 0.1 g larval tissue, so it was previously assumed that it would be necessary for a bird to eat many contaminated carcasses before reaching a titre of 100,000 MLD (oral LD_{50} for Delta Mallards on fresh water).

Warm water, driven over exposed mud banks and encrustations of "alkali" by wind tides or seiches (internal oscillations of lakes), can dissolve an increased amount of salts. A bird feeding and drinking in such a situation might eat several insect larvae and drink the water and so could ingest 20,000 to 30,000 MLD of the toxin. In itself, this is not sufficient to produce paralysis or death, but when taken in association with 20 cc or more of the highly saline water, it would be lethal.

One might suspect that death from botulism would occur more rapidly and symptoms would be more extreme as air temperature rises, but there are presently insufficient data to prove this point. However, since a functional salt gland effectively prevents dehydration of the individual possessing it, even partial malfunction of the gland could lead to dehydration. As air temperature increases, water requirements and the possibility of dehydration also increase. The chalky anal discharge noted in botulism victims may well be salts in extremely concentrated form and may represent the products of extreme physiological compensation for dehydration.

The fact that fresh water given a botulism victim in symptomatic stage 1 or 2 (see p. 385) is equally as effective as antitoxin appears to lend credence to the sequence of events outlined above. Force-fed fresh water would dilute concentrations of salts in the intestinal tract and extracellular

fluid and in turn provide extra fluids for excretion, via the kidneys, of salts already absorbed. If, on the other hand, the bird had ingested a massive dose of botulinum toxin, or was in symptomatic stage 3, fresh water had no effect and the bird died.

Workers studying botulism in the field have noted the irregular distribution of dead and dying birds. An area that appears ideal for an outbreak may be free of disease, while a similar area a short distance away may contain a large number of stricken birds. Such irregular distribution (especially in early stages of large outbreaks) may be a function of five variables: (1) distribution of invertebrates containing the bacteria; (2) distribution of pockets of highly alkaline or salty waters; (3) distribution of detritus capable of producing anaerobic conditions in the soil or water; (4) number of birds dispersing from loci of intoxication and dying elsewhere; and (5) number of birds arriving from an area of low salt concentration. Generally, outbreaks would occur when the first three variables were simultaneously favorable. Such a period may be short and investigation may occur after the damage has been done. As a result, it is unlikely that a worker could, except by chance, detect botulinum toxin in the environment in quantities sufficient to produce death by its actions alone.

Although the amount of toxin required to produce an LD_{50} varied little between Mallards and Pintails (when calculated on the basis of dose per kg body weight) in individuals of similar physical condition, experiments at Delta indicated that the physical well-being of a bird was important to its tolerance to a salt load or toxin, or both. A bird weakened by molt, growth, or disease was much more susceptible to toxin and salt water, given together or separately.

In Canada, most botulism outbreaks occur after the breeding season. The major outbreak at Whitewater Lake, Manitoba, in 1949 (Cooch, 1949) was typical of most Canadian outbreaks in timing (late July to early September), condition of birds (molting), and temperature (high 90's, Fahrenheit, during the day). Physical features of the lake fitted the classical concept of a botulism area—"feather edge" decomposing vegetation, alkaline waters, shoreline encrustations of alkali, wind tides, and exposed mud banks.

Kalmbach and Gunderson (1934), in considering Wetmore's (1915) theory of alkali poisoning, thought that birds would not drink highly alkaline waters. Further, they stated that 25,000 ppm dissolved alkali salts would be lethal to ducks. I found that no Mallards died after administration of 30 cc of 10 per cent NaCl and the subsequent *ad libitum* supply of 5 per cent NaCl solutions for periods of up to 10 days at Winnipeg and Delta. Thus the results of experiments reported by Kalmbach and Gunderson (1934) are difficult to interpret. It may be that some of the natural

"alkalis" they used were inherently toxic, a thesis developed by Shaw (1929), or that "fresh water ducks" with atrophied glands were involved in the observations of Kalmbach and Gunderson, and Shaw. Water taken from the nearly dry Louck's Pothole, of Delta, Manitoba, was given to Pintails and Mallards, and in both species subsequent nasal discharge was noted. When this water was given with amounts of *Clostridium botulinum* toxin below the LD_{50} level, death occurred. The control birds suffered no ill effects. Unfortunately, no means of analysis of the pothole water were available. However, the water exerted the same overt physiological effect, when measured by nasal discharge and lethality when given with the toxin, as did an equal volume of 5 per cent NaCl.

Kalmbach and Gunderson (1934) stated that birds would not drink highly alkaline waters and would go elsewhere to get "wholesome" water. However, Johnston Lake (Old Wives Lake), Saskatchewan, is a famous molting and botulism area. It is extremely alkaline, and during periods when it is nearly dry, dissolved salts exceed 100,000 ppm (Rawson and Moore, 1944). Birds molting there are trapped and thus must drink waters of very high salinity.

This report is preliminary in nature, and further investigations are planned to elucidate the resistance to botulism of birds moving from taiga to alkaline waters. It may well be that these migrants, because of their poorly developed extrarenal excretory capacity, are more susceptible to "botulism" than are resident birds.

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SUMMARY

Water birds inhabiting the Great Plains of North America possess a salt gland which may function as effectively as that of marine birds. The gland responds to increased osmolarity of blood plasma following ingestion of dissolved salts found in prairie waters. Thus a functional supraorbital gland has survival value for birds living in an alkaline environment.

Malfunction of the gland, caused by blockage of the facial nerve by pentobarbital sodium (Nembutal) or neurotoxin (Type C) liberated by *Clostridium botulinum*, can lead to death. I think that death in these birds from botulism can be a result either of a massive dose of toxin (probably unrelated to the salt gland) or of a minimal dose of toxin that causes decreased flow or cessation of flow (demonstrated) from the supra-orbital gland when this occurs in conjunction with a salt load or any other substance increasing osmolarity of the blood plasma.

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