perature and local reaction indicated the development of infection, the insulin injection and blood sugar determinations were repeated. Tests were made daily, usually for 2 or 3 days until the animal died or recovered.

Results. In general, similar results were obtained in all 6 animals. A typical experiment is illustrated in the text-figure. It appears that insulin administered during infection takes a little longer to produce its maximum effect, but the effect is more pronounced and lasts considerably longer. It also appears that the effect on reducing the blood sugar is in proportion to the gravity of the infection. In one animal, after insulin injection on the second day of infection the blood sugar dropped progressively to 78 mg. in 1¼ hours when death occurred. In another (temp. 41°C.) the level dropped even more rapidly. Convulsions occurred when the level dropped below 50 mg. and death followed about 30 minutes later with a level of 22 mg.

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Presence of Clostridium botulinum in Livers of Birds not Affected with Botulism.

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There has been some difference of opinion as to whether or not *Clostridium botulinum* possesses the ability to invade tissue. The identification of western duck sickness with botulism[1, 2] gives to this problem considerable hygienic importance, and the morbidity during an epizootic furnishes a sufficient number of birds to subject this problem to statistical analysis.

It has been observed[3] that cultures made from animals dying of lamsiekte contained botulinum in 50% of the cases, while 3% of the animals dying from other causes yielded such positives. During experiments by others regarding the ability of botulinum to pro-

[1] Kalmbach, E. R., *Science*, 1930, **72**, 658.

[2] Kalmbach, E. R., *Science*, 1932, **75**, 57.

[3] Scheuber, J. R., 15th An. Rep., S. A. Director of Veterinary Research, 1929, Part I, 223.

duce infection, some evidence indicates that even the detoxified spores can pass from the intestinal tract to the liver. However, the opinion has been expressed that *Cl. botulinum* does not usually invade the body, but that it may occasionally be recovered from the liver or spleen. In a discussion which also presents the literature,[4] experiments in which detoxified spores were fed and consequent deaths recorded are questioned on the basis that it is extremely difficult to free the spores completely from all traces of toxin. We believe that positive liver cultures produced by the ingestion of detoxified spores may be explained as due to the presence of minute traces of toxin. We have been unable to produce death in pigeons or ducks even with massive doses of detoxified viable spores.

Late in the fall of 1931, 34 ducks were examined after a lead-poisoning outbreak. Since the weather was cold, the dead birds were received in a frozen condition. The live birds were killed and autopsied immediately, the livers being removed aseptically. Toxicity tests and typing experiments showed *Cl. botulinum* Type C to be present in one of the liver cultures, while Type A was found in 2 cases, one of which was a duck received alive and killed immediately before necropsy.

During the fall of 1932, 2 botulinum cultures were obtained from a group of 5 ducks received from the Minnesota State Conservation Department. One culture was Type B; the other contained Types A and C.

Thirty-one livers were cultured from normal grouse, that had been shot, but the shot had not penetrated the viscera. One culture was positive for *Cl. botulinum,* Type A.

These results indicate that birds can harbor the botulinum organism, the presence of which possibly represents a previous exposure to toxic material from which the bird recovered. The difference between grouse and ducks probably represents variations in the toxin hazards of their respective environments.

Aside from the possibility that the presence of *Cl. botulinum* in such birds constitutes a potential danger in game birds as human food, if handled carelessly, the fact that such botulinum "carriers" provide a means for the dissemination of the organism is of greater importance. Migratory birds feeding in enzootic duck-sickness areas could thus increase the amount of soil infection in other favorable areas if they should die elsewhere and release *Cl. botulinum,* Type C, from their bodies. Types A and B could be carried like-

[4] Topley and Wilson, The Principles of Bacteriology and Immunology. William Wood and Co., New York. 1929.

wise. In birds cultured from Minnesota, Type A is most frequently encountered, although in the case of the ducks it can not be stated with certainty that the organism recovered was picked up in Minnesota. The presence of *Cl. botulinum* in the livers of various members of the wild-life population of an area would constitute a valuable criterion as to the presence of *Cl. botulinum* in soil. The types of botulinum recovered from the livers of normal ducks representing the principal migratory routes followed in the United States would yield data of significance as to the botulinum infestation in the 3 areas.

The results obtained from the cultures just discussed will be compared with data obtained by use of the pigeon as the experimental subject. Such data make up a portion of a more complete study to be presented elsewhere.[5]

The occurrence of *Cl. botulinum* in the livers of normal pigeons was investigated. Twenty pigeons were killed and their livers removed aseptically for culture. Injections to determine toxicity yielded negative results. The failure to find organisms in the livers of normal pigeons from this region is not surprising, since *Cl. botulinum* is not a common soil inhabitant here, and the food habits of pigeons render them less likely to come in contact with grossly contaminated food than is the case with ducks.

One cubic centimeter of Type C culture, detoxified by heating at 80°C. for 30 minutes, was fed to each of 6 birds. A culture made from such detoxified spores was toxic upon injection after a period of incubation, thus indicating that the spores were viable. Forty-eight hours after spore ingestion, the birds were decapitated and liver cultures were made. *Cl. botulinum,* Type C, was present in one of the cultures.

These results verify those obtained from cultures of livers from natural sources. *Cl. botulinum* may be present in the livers of birds neither sick nor dead of botulism, and negative results may be obtained from cultures of birds dying of botulism.

Evidence which it may be possible to substantiate indicates that time is required for the organism to pass through the intestinal tract and reach the liver. In 2 of 3 cases of an intoxication of 9 hours' or less duration with Type C cultures, no organisms were detected in liver cultures. This may indicate that, in the case of a disease with a rapidly fatal termination, negative cultures may be expected.

[5] Kalmbach, E. R., and Gunderson, M. F. Unpublished data in the Bureau of Biological Survey on "Western Duck Sickness; a Form of Botulism." (Being prepared for publication by the U. S. Department of Agriculture.)

TABLE I.

Type of botulinum recovered from liver cultures made from birds fed 1 cc. of Type A or C culture; effect of such feeding upon the birds. The dosage lethal for pigeons by injection was 0.001 cc. for the Type C, and 0.0001 cc. for the Type A culture.

Bird No.	Duration of disease	Results of typing experiments on liver cultures
	Birds fed Type C	
191	8 hr.	Negative
192	36 hr.	Type C
193	12 hr.	" "
194	9 hr.	Negative
195	12 hr.	Type C
196	Unaffected; killed after 144 hr.	" "
197	8 hr.	" "
198	18 hr.	" "
199	Lethargic; killed after 144 hr.	" "
200	Unaffected, killed after 144 hr.	" "
	Birds fed Type A	
578	12 hr.	Negative
579	12 hr.	Type A
580	30 hr.	" "
581	Unaffected; killed after 144 hr.	Negative
582	24 hr.	Type A
583	Unaffected; killed after 144 hr.	Negative
584	Unaffected; killed after 144 hr.	Type A
585	48 hr.	Negative
586	Unaffected; killed after 144 hr.	"
587	Unaffected; killed after 144 hr.	"

It is also postulated that passage or non-passage of the organism from the lumen of the intestine to the liver is dependent upon some factor that alters the permeability of the intestinal tract. Whether any intestinal disturbance coincident with the ingestion of detoxified spores would cause the appearance of organisms in the liver or whether the botulinum toxin is the controlling factor is also under investigation.