coast. The primary purpose of the expedition is to obtain information on the developmental stages of what may be a modern geosyncline. The oceanographic work by Roger Revelle and F. P. Shepard will produce data regarding the environment of deposition of the marine Tertiary, especially the Miocene diatomaceous shales, rate of accumulation of diatomaceous muds, causes of the laminations in them and significance of their organic content as a possible source of petroleum, information on the tectonics of the area with possible data bearing on the southward continuation of the San Andreas fault system, and new information on submarine canyons. The land exploration will be carried out by W. S. W. Kew and C. A. Anderson in virtually unexplored territory difficult of access and of unusual interest. Studies of the marine Tertiary and of the thick interbedded volcanics should yield information on the structure and earlier development of the great depositional basin. $2,500.

G. E. Condra, University of Nebraska, with the assistance of M. K. Elias, will continue the photographing and description of his large collections of Pennsylvanian and Permian Bryozoa in the preparation of monographic reports. Through many years Professor Condra has collected systematically through the Mid-Continent region and in New Mexico, Arizona, Utah, Wyoming and South Dakota. He has collected in Russia and studied the collections in Perm, Moscow, Leningrad, Stockholm, Copenhagen and London. The acquisition of material from the Glass Mountains of Texas and from Greenland has made the Nebraska collection the largest in the world. Work is already three quarters complete and will be continued through the remainder of the year. $1,400.

SPECIAL ARTICLES

THE SUSCEPTIBILITY OF CHICKS TO DIPHTHERIA BACILLI AND TOXIN

IN 1884 Loeffler[1] described the effect of introducing virulent diphtheria bacilli into various birds and animals. Guinea pigs and rabbits were found to be especially susceptible. Later workers abundantly confirmed the latter findings of Loeffler, and these two animals were adopted, to the virtual exclusion of all others, in the study of diphtheria. From the work of Behring, Erlich and others upon measurements of toxin and antitoxin, the 250-gram guinea pig became the standard for such purposes and remains so to-day.

Among other tests made by Loeffler were those on several species of birds. Of thirteen finch-like birds or canaries inoculated intramuscularly with different strains of diphtheria bacilli, all died and at autopsy the organisms were found in large numbers in the tissues immediately surrounding the site of inoculation. All but one of eleven pigeons inoculated by various routes also succumbed to the infection. The effect of inoculating chickens ("fowls") intratracheally was less definite, although membrane-formation in some was observed. In 1899 Salter[2] reported that typical diphtheria bacilli were pathogenic for sparrows, finches, yellow-hammers and the thrush, on the basis of a small number of observations. The studies of Loeffler and Salter on birds were apparently never pursued further.

While retesting the pathogenicity of the diphtheria group of organisms for various species of birds and animals, it was noted that 7-day-old chicks regularly succumbed, following subcutaneous or intraperitoneal inoculation of strains of *Corynebacterium diphtheriae* known to be virulent for rabbits. Subsequent studies showed that death of the chicks rarely followed the inoculation of rabbit-avirulent diphtheria bacilli or diphtheroids. Moreover, as little as one guinea-pig-M.L.D. of diphtheria toxin regularly killed the chicks, while a high degree of protection was afforded by a previous injection of diphtheria antitoxin. Brief details of these experiments follow.

The cultures of *C. diphtheriae* were isolated during the course of local and nation-wide surveys, in which over 2,000 strains of diphtheria bacilli[3,4] were obtained from healthy carriers and persons with diphtheria. All these strains had previously been examined in this laboratory, and tested for virulence by the intracutaneous method in rabbits, developed by Fraser and Weld.[5] Strains referred to in this study will be designated as "virulent" or "avirulent" on the basis of that test. The organisms used in the experiments described in this paper were incubated for 48 hours in infusion-broth; 0.5 cc being the usual dose.

In a preliminary experiment, week-old White Leghorn or Barred Plymouth Rocks were inoculated subcutaneously in the back near the insertion of the right wing with six different strains of virulent organisms, among which were strains of the gravis, mitis and intermediate types. Of eighteen chicks inoculated, all died within 48 hours, regardless of type of organisms or breed of chick used. Six chicks, previously injected intraperitoneally with 100 units of diphtheria antitoxin, when inoculated with the same six virulent cultures, survived with no evident illness. Likewise, four chicks inoculated with four different strains of avirulent *C. diphtheriae* remained well.

[1] F. Loeffler, *Mitth. a. d. k. Gsndhtsamte.*, 2: 421–99, 1884.

[2] A. Salter, *Trans. Jenner Inst. Prev. Med.*, London, 2 s: 113, 1899.

[3] W. H. Frost, M. Frobisher, Jr., V. A. Van Volkenburgh and M. Levin, *Am. Jour. Hygiene*, 24: 568–586, 1936.

[4] M. Frobisher, Jr., *Supplement to Am. Jour. Pub. Health*, 30: 28–35, No. 3, 1940.

[5] D. T. Fraser and C. B. Weld, *Trans. Roy. Soc. Canada*, (Sect. V) 20: 343, 1926.

In another experiment with seven-day-old White Leghorn chicks, the following results were obtained: (1) Each of twelve strains of virulent organisms was inoculated subcutaneously in one chick; all twelve chicks died within 24 hours. (2) Twelve chicks, injected intraperitoneally with 200 units of antitoxin two hours previously, were inoculated subcutaneously with the same twelve strains; all twelve chicks remained well for two weeks or longer. (3) Of twelve chicks inoculated with twelve different strains of avirulent *C. diphtheriae,* all but one remained well for two weeks. (4) Of ten chicks inoculated with ten different cultures of diphtheroids, all remained well.

The protocol of a similar experiment with strains other than those mentioned above is shown in Table 1.

TABLE 1

EFFECT OF VARIOUS STRAINS OF CORYNEBACTERIA IN CHICKS

Organism	Result of virulence test in rabbit	Strains	Chicks	Dead	Paralyzed	No visible reaction
C. diphtheriae (with antitoxin)	Positive	10	20	4†	0	16
C. diphtheriae (no antitoxin)	Positive	10	20	19	1	0
C. diphtheriae	Negative	10	20	1*	0	19
C. xerosis	Negative	10	20	1	0	19
C. pseudodiphthericum	Negative	10	20	0	0	20

† The other chick of these pairs, in each instance, survived with no visible illness.
* Only after 5 days; no paralysis observed.

Two White Leghorn chicks were inoculated with each strain. The results are essentially the same as those obtained in the previous experiments.

Altogether, of fifty unprotected chicks injected with 35 different strains of rabbit-virulent *C. diphtheriae,* only one survived, and this after a severe illness, while, of 38 antitoxin-protected chicks injected with the same strains, 33 survived and 5 died, 3 of them from nonspecific causes. Of 36 chicks injected with 26 strains of avirulent *C. diphtheriae,* 34 survived and 2 died. In addition, of fifty chicks receiving thirty strains of diphtheroids, both *C. xerosis* and *C. pseudodiphthericum,* all but one survived with no apparent illness. The possibility of using chicks as test subjects in determining the virulence of cultures of *C. diphtheriae* is thus clearly indicated.

Results obtained with toxic filtrates were equally definite. A preliminary experiment has shown that chicks succumb to *intraperitoneal* injection of as little as 0.5 guinea-pig-M.L.D. of toxin. In Table 2 details are given concerning a group of 48 chicks receiving five different doses of toxin. The data indicate a susceptibility approximating that of 250-gram guinea pigs. In those chicks designated as "ill," paralysis of the wings and legs was a prominent feature. The chicks given doses of toxin larger than 1.0 M.L.D.

TABLE 2

EFFECT OF VARIOUS DOSES OF DIPHTHERIA TOXIN UPON SEVEN-DAY-OLD WHITE LEGHORN CHICKS

Dose of toxin (guinea pig M.L.D.s)	Chicks injected	Fate
3.0	6	6 dead within 26 hours
1.5	12	11 dead within 40 hours 1 very ill for 36 hours, survived, later showed paresis
1.0	13	6 dead within 72 hours 3 " " 5 days 2 ill for 2 days, survived, later showed paresis 2 no evident illness
0.5	13	2 dead within 72 hours 3 " " 5 days 2 moribund and killed on fifth day 1 dead after 6 days 3 ill for 2–3 days, survived, later showed paralysis 2 no evident illness
0.1	6	3 slightly ill up to 96 hours, survived, no damage 3 no evident illness

died promptly. Those receiving less than 1.0 M.L.D. also sometimes died or were ill, but the effect of the toxin was definitely delayed, and paralysis was sometimes not manifest for 36 hours. The contrast between 1.0 M.L.D. and 0.5 M.L.D. in this respect, for example, was quite evident. The reaction to a given dose seemed to be more uniform in the chick than is the case with guinea pigs.

Thus, it has been shown that seven-day-old White Leghorn chicks succumb regularly within 48 hours to subcutaneous injections of 48-hour infusion-broth cultures of strains of *C. diphtheriae* which are virulent for rabbits. The chicks are rarely, if at all, affected by similar infections if protected by intraperitoneal injections of at least 200 units of antitoxin given 2 hours before. Cultures of *C. diphtheriae* and of diphtheroids, previously shown to be avirulent for rabbits, have no perceptible effect on the chickens.

Further, it has been demonstrated that week-old White Leghorn chicks have about the same degree of susceptibility to diphtheria toxin as 250-gram guinea pigs.

These experiments clearly indicate the value of the chick in the study of the diphtheria group of organisms. It is possible that both virulence tests and tests for the potency of diphtheria toxin can be satisfactorily made in week-old chicks. The economic advantages are obvious.[6]

MARTIN FROBISHER, JR.
THE JOHNS HOPKINS SCHOOL OF HYGIENE AND PUBLIC HEALTH

THE ANTI-TRYPTIC PROPERTIES OF HEPARIN

INVESTIGATIONS of the possible prolongation of insulin action by inhibitors of proteolytic activity[1] have

[6] The author takes pleasure in acknowledging, with appreciation, the technical assistance of Dr. Elizabeth I. Parsons and the advice and encouragement of Dr. Thomas B. Turner.