

Susceptibility of the Eastern Cotton Rat (*Sigmodon hispidus littoralis*) to Diphtheric Toxin.

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The high degree of natural resistance of the albino rat (*Mus norvegicus albinus*) to diphtheric infection and intoxication is a matter of common knowledge. While there may be some uncertainty as to the precise amount which rats will tolerate, all investigators agree that many times the lethal dose for guinea pigs of toxin or of virulent bacilli can be injected into rats without causing death.¹ Armstrong² reported that a variety of Eastern cotton rats (*Sigmodon hispidus hispidus*) are apparently susceptible to infection with poliomyelitic virus. There are no data on the reaction of the cotton rat to diphtheric infection or intoxication. In view of a number of previous observations, all of which suggest some relationship between susceptibility to diphtheria and to poliomyelitis,³ our interest was aroused to determine whether these rodents are as refractory to experimental diphtheria as is the albino rat.

The cotton rats were obtained in several shipments from a dealer in Florida who had trapped the wild animals in the field. They appeared to be a variety of Eastern cotton rats, listed as *Sigmodon hispidus littoralis*, which is closely related to *Sigmodon hispidus hispidus*. They were sturdy specimens, weighing from approximately 70 to 120 g, and thrived when placed on a diet consisting of oatmeal, peanuts, green vegetables and apples. However, upon necropsying animals that had died as the result of experimentation, it was found that they were, as a rule, heavily infected with intestinal and pulmonary worms and occasionally carried trypanosomes in their blood. The latter observation will make the subject of another

¹ Diphtheria Monograph, Medical Research Council, London, 1923.

² Armstrong, C., *Publ. Health Rep.*, 1939, **34**, 1719.

³ (a) Zingher, A., *Am. J. Dis. Childr.*, 1917, **13**, 247; (b) Jungeblut, C. W., *J. Immunol.*, 1934, **27**, 17; (c) Foley, A. R., *Canad. Publ. Health J.*, 1934, **25**, 260; (d) Jungeblut, C. W., *Am. J. Med. Sci.*, 1936, **192**, 661; (e) Fischer, A. E., and Stillerman, M., *Am. J. Dis. Childr.*, 1937, **54**, 984; (f) Dwyer, J. M., *Med. J. Australia*, 1938, January 8, p. 52; (g) Heaslip, W. G., *Austral. J. Exp. Biol. and Med. Sci.*, 1938, **16**, 285; (h) Burnet, F. M., Freeman, M., Jackson, A. V., and Lush, D., *Med. J. Australia*, 1939, August 5, p. 198.

TABLE I.
Infection with *C. diphtheriae* in the Guinea Pig, Cotton Rat, and Albino Rat.
(Subcutaneous injection of bacillary suspension.)

<i>C. diphtheriae</i> , <i>gravis</i> strain	Guinea pigs No.	Result		Cotton rats No.	Result		Albino rats No.	Result	
		Died	Lived		Died	Lived		Died	Lived
1 slant	1	1 (24h)	0	1	1 (48h)	0	1	0	1
1/2 "	1	1 "	0	1	1 (4d)	0	1	0	1
1/5 "	1	1 "	0	1	1 "	0	1	0	1
1/10 "	1	1 (48h)	0	2	2 (4d, 6d)	0	2	0	2
1/20 "	1	1 "	0	1	1 (5d)	0	1	0	
1/40 "	1	1 "	0	1	0	1	1		
1/100 "	1	0	1	1	0	1			
1/200 "	1	0	1						

detailed report by Dr. Culbertson from this laboratory. We are mentioning these facts briefly at this time since they may possibly have a bearing on the resistance of this animal to experimentally induced infections and intoxications. So far no laboratory-bred cotton rats, free from intercurrent disease, have been available.

Diphtheric infection: A strain of *C. diphtheriae* of known high virulence was used for this work (London *gravis* I). We are indebted to Dr. Martin Frobisher, Jr., of Johns Hopkins University for kindly sending us this strain. Cultures grown for 24 hours on Loeffler slants in 4" x 1/2" tubes were suspended in saline and the bacterial emulsion, in graded doses, was injected subcutaneously into guinea pigs, cotton rats, and albino rats. The results are given in Table I. It appears from these tests that amounts as small as 1/40 of a slant killed guinea pigs uniformly within 48 hours, whereas 1/20 of a slant sufficed to cause death in the cotton rat within 5 days. No deaths occurred in a series of albino rats injected with amounts varying from 1/10 of a slant to as much as a full slant. The lesions in guinea pigs, both local and in the adrenal, were pathognomonic of diphtheric death. A similar characteristic local lesion consisting of a gelatinous, hemorrhagic exudate was but rarely obtained in the cotton rat; more often there was evidence of a localized abscess at the point of in-

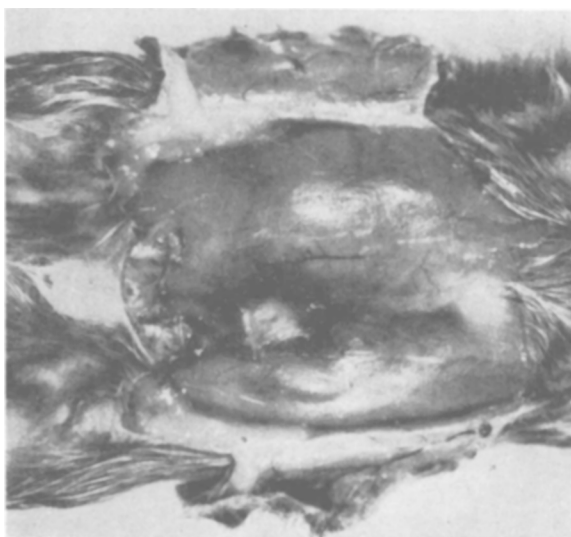


FIG. 1.

Local lesion in the cotton rat following subcutaneous injection of culture of *C. diphtheriae*.

jection which yielded a pure culture of diphtheria bacilli upon transfer to suitable media. This lesion is well illustrated in photograph 1. Adrenal involvement occurred irregularly and usually was not very intense. Such abscesses as have just been described were regularly seen in surviving albino rats when these animals were sacrificed during the early intervals following infection. Systematic observations on the type of cellular response of the albino rat to diphtheric infection and subsequent changes in the morphology and virulence of the bacilli will be reported elsewhere.

Diphtheric intoxication: The toxin employed was a sample of Park-Williams No. 8 diphtheric toxin and was obtained through the courtesy of the New York City Department of Health. At the time of running these tests, the minimal fatal dose for guinea pigs was 1 cc of a 1:700 dilution. Doses of toxin ranging from 2000 mfd to 1 mfd were injected subcutaneously into guinea pigs, cotton rats, and albino rats. The results are brought together in Table II. It will be seen from the figures that cotton rats succumbed uniformly to amounts of toxin as small as 5 mfd; further reduction to 2 mfd caused the death of 2 out of 4 cotton rats, while a similar number of animals survived a single fatal dose for the guinea pig. As might have been expected, albino rats tolerated inordinately larger amounts of toxin, succumbing only to 1000 and 2000 mfd. In harmony with the results obtained with bacillary infection, the fully developed picture of the local lesion, which is so characteristic for guinea pigs, was rarely seen in the cotton rat; moreover, adrenal congestion usually was not very marked and was occasionally absent. Despite prolonged observation, surviving cotton rats never exhibited any signs of postdiphtheric paralysis, excepting one animal which showed flaccid paralysis of both hind legs 6 weeks following injection with 1 mfd of toxin; this animal died 2 days later.

The above results clearly indicate the high systemic susceptibility of the cotton rat to diphtheric intoxication. The objection might be raised that some of our animals were abnormally sensitive to the toxin because a certain percentage were simultaneous carriers of intestinal parasites and of trypanosomes. Such an assumption, however, would hardly explain the closely graded correspondence, throughout the entire experimental series, between the size of the inoculum—be it toxin or culture—and the response of the animal, as measured by the length of its survival. In order to throw more light on this problem another experiment was carried out in which we studied the local effect of the toxin following endermal injection in

TABLE II.
Effect of Diphtheric Toxin Injected Subcutaneously into Guinea Pigs, Cotton Rats, and Albino Rats.

Diphtheria Toxin, mfd	Guinea pigs, No. of animals	Result		Cotton Rats No.	Result		Albino Rats No.	Result	
		Died	Lived		Died	Lived		Died	Lived
2000								2 (5d, 6d)	0
1000								2 "	0
700				1	1 (24h)	0		2 "	2
350				1	1 "	0		0	0
200				1	1 (36h)	0		0	0
100				2	2 (36h, 36h)	0		0	2
50				2	2 (60h, 72h)	0		0	2
25				3	3 (72h, 72h, 72h)	0		0	2
10				3	3 (72h, 5d, 5d)	0		0	2
5				4	4 (5d, 8d, 8d, 10d)	0		0	2
2				4	2 (8d, 8d)	2		0	2
1*	4	4 (48h, 48h, 72h, 96h)	0	4	0	4†			

*Represents 1 cc of a 1:700 dilution of toxin.

†See text for development of post-diphtheric paralysis and late death in one animal.

TABLE III.
Effect of Diphtheric Toxin Injected Endermally into Guinea Pigs, Cotton Rats, and Albino Rats.

Animal	Interval	Mfd of toxin injected endermally, each dose being contained in a uniform volume of 0.1 cc									
		1	1/2	1/5	1/10	1/50	1/100	1/200			
Guinea Pig No. 1	24h				+	+	+	+	+	+	+
	72h				+	+	+	+	+	+	+
	5d				+	+	+	+	+	+	+
" " No. 2	24h				+	+	+	+	+	+	+
	72h				+	+	+	+	+	+	+
	5d				+	+	+	+	+	+	+
Cotton Rat No. 1	24h				0	0	0	0	0	0	0
	72h				0	0	0	0	0	0	0
	5d				0	0	0	0	0	0	0
" " No. 2	24h	0	0	0	0	0	0	0	0	0	0
	72h	+	+	+	+	+	+	+	+	+	+
	5d	+	+	+	+	+	+	+	+	+	+
" " No. 3	24h	0	0	0	0	0	0	0	0	0	0
	72h	±	0	0	0	0	0	0	0	0	0
	5d	+	+	±	±	±	±	±	±	±	±
Albino Rat No. 1	24h	0	0	0	0	0	0	0	0	0	0
	72h	0	0	0	0	0	0	0	0	0	0
	5d	0	0	0	0	0	0	0	0	0	0
" " No. 2	24h	0	0	0	0	0	0	0	0	0	0
	72h	0	0	0	0	0	0	0	0	0	0
	5d	0	0	0	0	0	0	0	0	0	0

0 = no reaction.

± = questionable reaction.

+ = slight redness.

++ = marked redness.

+++ = slight necrosis.

++++ = ulceration.



FIG. 2.

Local lesions in the cotton rat following endermal injection of 1 and 1/2 mfd of diphtheric toxin.

guinea pigs, cotton rats, and albino rats. The results are given in Table III. It will be seen that cotton rats failed to react to threshold doses of toxin, such as 1/10 and 1/50 mfd, which produce severe lesions on the guinea pig's skin. However, comparable skin-reactions, consisting of initial induration and redness followed by necrosis and ulceration, were readily obtained in cotton rats by the injection of

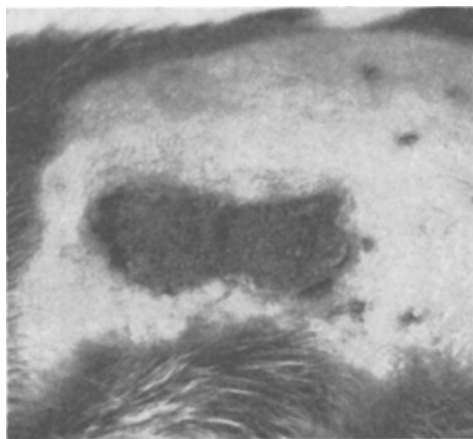


FIG. 3.

Skin-reaction with 1 and 1/2 mfd of diphtheric toxin in the cotton rat. (Fig. 2 enlarged).

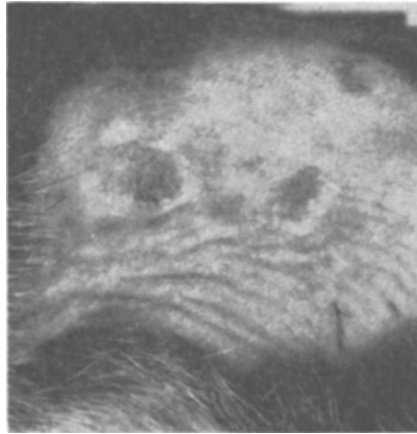


FIG. 4.

Skin-reaction with 1/5 and 1/10 mfd of diphtheric toxin in the cotton rat.

one or $\frac{1}{2}$ mfd. These reactions are clearly shown in photographs 2 to 4. In keeping with previous experience, no skin-reactions were observed in albino rats with any of the doses of toxin included in our range.

Conclusions. The results reported in this paper leave no doubt that the Eastern cotton rat is markedly susceptible to diphtheric infection and intoxication. While not equalling the extraordinary susceptibility of the guinea pig, the cotton rat appears to be at least many times more susceptible than the highly resistant albino rat. The significance of this observation in relation to the zoölogical classification of the cotton rat among the group of rodents remains to be determined.