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A Toxic Substance Associated with the Gilliam Strain of *R. orientalis.*

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A substance toxic for mice occurs in yolk sac tissues infected with *R. mooseri*[1] and *R. prowazeki.*[2] These related toxins are neutralized by typhus antisera,[1,2] but are not immunologically identical.[3,4] The present report describes the occurrence of a specific toxic material in yolk sacs infected with the Gilliam strain of *R. orientalis.*

Materials and Methods. The Gilliam strain,[5] received from Dr. N. H. Topping, was maintained by serial passage in yolk sacs of 7-day-embryonated eggs which were incubated at 35°C after inoculation. Yolk sacs infected with the 25th to 36th egg passage yielded toxic material. The methods of preparation and testing were as follows: Sacs of embryos moribund on the sixth to ninth day after inoculation were harvested, and smears, stained by a modified Macchiavello's technic, were examined microscopically. Those containing 25-100 recognizable rickettsiae per field were pooled and a 20% suspension prepared in sterile skimmed milk. Following low speed centrifugation, ½, ¼ and ⅛ dilutions of the supernatant fluid were prepared in 0.9% NaCl solution containing 10% normal rabbit serum. 0.5 cc of each dilution was injected intravenously into each of 5 mice. Deaths were recorded up to 18 hours when survivors were discarded. Toxin-neutralization tests were done as follows: A ½ dilution of a 20% suspension of freshly harvested infectious sacs was tested for toxin in 5 mice; if 4 died within 2 hours, the material was considered suitable. Serial 2-fold dilutions of sera to be tested were then prepared in 10% normal serum-NaCl solution. These were mixed with equal volumes of the stock 20% suspension of sacs which had been stored meanwhile at 0°C. Intravenous injection of mice with 0.5 cc amounts of the mixtures was begun immediately and usually completed within an hour. A control titration of the toxic suspension was performed at the end of the test. Toxin-neutralization tests were done with pooled sera of guinea pigs and serum of one patient recovered from infection with the Gilliam strain, and with pooled sera of rabbits hyperimmunized with splenic tissue of mice dead from infection with this strain. Antisera against the Karp,[5] Kostival,[6] Host 21,[6] Seerangayee,[7] Volner,[8] Imphal,[9] Wild Rat No. 2,† and CBI Rat† strains of scrub typhus were also tested for antitoxin. These had been shown in other experiments[10] to be capable of protecting mice from death caused by infection[11] with the homologous strain.

Results. Mice injected intravenously with toxic preparations appeared normal for 1 to 1½ hours, then developed dyspnea and weak-

* Member of United States of America Typhus Commission.

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TABLE I.
Neutralization of Toxin of *R. orientalis* (Gilliam Strain) by Hyperimmune and Convalescent Scrub Typhus Sera.

Toxin		Antisera			Results	
					Death of mice*	Antitoxin titer (dil. of serum)
Material	Dilution	Strain	Source	Dilution		
	1/10	None			17/20	
	1/20	,,			1/10	
	1/40	,,			0/5	
	1/10	Gilliam	rabbit	1/10	0/5	
	,,	,,	,,	1/40	0/5	
	,,	,,	,,	1/160	0/5	1/640
	,,	,,	,,	1/640	0/5	
	,,	,,	guinea pig	1/10	0/5	
	,,	,,	,,	1/40	1/5	
	,,	,,	,,	1/160	0/5	1/640
	,,	,,	,,	1/640	0/5	
	,,	,,	human	1/5	1/5	
	,,	,,	,,	1/10	0/5	1/20
	,,	,,	,,	1/20	0/5	
Gilliam yolk sac 31st passage	,,	Karp	guinea pig	1/10	1/5	
	,,	,,	,,	1/20	0/5	1/40
	,,	,,	,,	1/40	0/5	
	,,	Wild rat No. 2	rabbit	1/10	1/4	
	,,	,,	,,	1/20	2/5	1/10
	,,	,,	,,	1/40	5/5	(partial 1/20)
	,,	Imphal	,,	1/10	1/5	
	,,	,,	,,	1/20	3/5	1/10
	,,	,,	,,	1/40	4/5	(partial 1/20)
	,,	Volner	,,	1/10	5/5	
	,,	,,	,,	1/20	4/5	Negative
	,,	,,	,,	1/40	5/5	
	,,	CBI rat	,,	1/10	5/5	
	,,	,,	,,	1/20	5/5	,,
	,,	,,	,,	1/40	3/5	
	,,	None			11/15	
	1/20	,,			9/15	
	1/40	,,			0/15	
	1/10	Gilliam	rabbit	1/320	0/5	
	,,	,,	,,	1/640	0/5	1/640
	,,	,,	,,	1/1280	2/5	(partial 1/1280)
Gilliam yolk sac 36th passage	,,	Seerangayee	,,	1/10	0/5	
	,,	,,	,,	1/20	4/5	1/10
	,,	,,	,,	1/40	4/5	
	,,	Host 21	guinea pig	1/10	5/5	
	,,	,,	,,	1/20	4/5	Negative
	,,	,,	,,	1/40	4/5	
	,,	Kostival	rabbit	1/10	4/5	
	,,	,,	,,	1/20	5/5	,,
	,,	,,	,,	1/40	5/5	

* Numerator indicates number of mice which died and denominator indicates the number of mice in each group.

ness followed rapidly by prostration, cyanosis, convulsions and death. Practically all deaths occurred within 3 hours. Certain mice affected by the toxin recovered and looked healthy for several days; all subsequently died in 4-6 days with the usual signs of infection[9] and with numerous rickettsiae in their spleens and lungs.

The toxic factor occurred with some regularity in our preparations although the titers were never high. In 21 experiments 5 suspensions killed the majority of mice receiving a 1/20 dilution and 8 killed at 1/10, while 8 were non-lethal at the latter dilution. The toxicity of a suspension generally diminished after freezing and storage at —70°C or —20°C for a few hours but was consistently unaffected by storage at 0°C for 3-4 hours. These same general methods have failed in our hands to demonstrate toxicity in yolk sacs heavily infected with the Imphal, Calcutta,[9] Karp, Kostival and Host 21 strains of *R. orientalis*. Whether this indicates inherent differences in these strains or that growth is not profuse enough in eggs to produce detectable toxin remains to be determined.

Sera of normal men, rabbits and guinea pigs did not neutralize the toxin. In contrast, a 1/640 dilution of anti-Gilliam rabbit or guinea pig serum neutralized a lethal dose of toxin, and the patient's serum had a titer of 1/20, Table I. Antisera against only 4 of the 8 heterologous strains of *R. orientalis* contained even small amounts of antitoxin, yet each serum was known to protect mice against death by infection with at least 1,000 lethal doses of its homologous strain. Thus, results of toxin-antitoxin tests contribute to the other types of evidence[5,11] regarding immunological differences among strains of *R. orientalis*. Sera of guinea pigs convalescent from epidemic typhus, murine typhus, or spotted fever failed to neutralize the Gilliam toxin; their specific complement fixing titers[12] were 1/240, 1/320, and 1/320, respectively.

Conclusion. Yolk sacs of embryonated eggs infected with the Gilliam strain of *R. orientalis* contain a specific toxin lethal for mice. The toxic substance is readily neutralized by Gilliam antiserum, but antisera against 8 other strains of *R. orientalis* contain only small amounts of antitoxin or none.

[12] Plotz, H., Wertman, K., and Bennett, B. L., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 76.

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Effect of Distention of Jejunum upon Tonicity of the Cardia of the Dog.

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This study was undertaken to determine if there are any extrinsic reflex effects upon the tonus and motility of the cardia during distention of the jejunum.

Methods. Records were obtained from 4 dogs over a period of 3 months. A modification and extension of the method outlined by Zeller and Burget[1] for recording motility of the cardia in the unanesthetized dog was used. A jejunal Thiry fistula was prepared in each dog, then an esophagostomy was made following recovery from the first operation.

About a week after the esophagostomy was performed, the training of the dogs was begun. They were trained to lie quietly on a table with the balloons in position while records of motility were obtained. Three balloons were in place and simultaneous tracings made on a smoked drum. The upper balloon was a 2 cm segment of condom tied over a rather rigid 14 Fr. catheter and inserted about 4 or 5 cm into the esophageal fistula and tied in place. It was attached to a

[1] Zeller, W., and Burget, G. E., *Am. J. Dig. Dis.*, 1937, **4**, 113.