BRIEF NOTE

Virulence of *Salmonella typhimurium* Harbored in the Feces of Carrier-Dog for Mice

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In the previous paper the authors reported [6] that almost all the dogs given orally a small amount of *S. typhimurium* harbored in the feces of canine carrier ("the organisms in feces") were found to be infected latently with the organism producing carrier state, while none of the dogs administered with a small amount of the broth culture of a fresh isolate of the serovar from the feces ("fresh isolate") acquired the infection. These findings indicate that there is a remarkable difference in the infectivity against dogs between "the organisms in feces" and "fresh isolate".

In this experiment, 3 different inocula prepared for *S. typhimurium*, such as "the organisms in feces", "fresh isolate" and a fresh isolate mixed with *Salmonella*-free dog feces ("fresh isolate mixed with feces") were given orally to 6-week-old female mice, and the response of the mice was observed for 14 days. The mice used were of the NIH (gpc) strain free of spontaneous *Salmonella* infection, and were raised in this Institute. Of 11 groups consisting of 5 mice each, 3 were administered with 54 to 92 cells of "the organisms in feces", 3 with 5 to 555

cells of "fresh isolate", and 4 with 6 to 6,000 cells of "fresh isolate mixed with feces". The remaining one group was given *Salmonella*-free dog feces as a control. Each group of mice was housed in a metal cage with wood shaving sterilized by autoclaving, and the cage was replaced daily. The pelleted food given to the mice was confirmed to be free from *Salmonella* contamination. The "fresh isolate" was prepared according to the method described previously [6], except for that no skim milk powder was added. As for "the organisms in feces", 10% suspension of carrier-dog feces was made in infusion broth (3–5 g/30–50 ml) from horse meat, and kept at 37°C for 30 min. After filtration through 3 layers of gauze, the filtrate was centrifuged at 4,000 rpm for 30 min. One part of the sediment was resuspended in 9 parts of the broth. For preparation of "fresh isolate mixed with feces", *Salmonella*-free dog feces obtained from a breeding colony in this Institute was treated in the same manner as mentioned above, and 10 ml of suspension was added to 0.1 ml of an appropriate dilution of "fresh isolate" with the infusion

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Table 1. *Salmonella* shedding in mice administered with *S. typhimurium* harbored in the canine feces

Dose* (cells)	No. of mice	Days after administration													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
54	5	—	—	+	+	+	—	—	+	+	+	+	+	+	+
										(d)	(d)				
54	5	—	+	+	—	+	+	+	+	—	+	+	—	—	+
								(d)			(d)		(d)		
92	5	—	—	—	+	—	—	+	+	+	+	+	+	+	—
												(d)	(d)		

Remarks.

*: Number of *S. typhimurium* given to a mouse.(d): A mouse died of sepsis by *S. typhimurium*.Table 2. The formation of gross lesion, recovery of organism and antibody titer in mice administered with *S. typhimurium* harbored in the canine feces

No. of mice	Gross lesion*			Recovery of organism								Antibody titer**	
	Li	M.L.	S	H.B.	Li	S	M.L.	Lu	K	C	I	O	H
7 Died within 7-12 days	+	+	+	+	+	+	+	+	+	+	•	•	•
	+	+	+	+	+	+	+	+	+	+	•	•	•
	+	+	+	+	+	+	+	+	+	+	+	•	•
	+	+	+	+	+	+	+	+	+	+	•	•	•
	+	+	+	+	+	+	+	+	+	+	•	•	•
	+	+	+	+	+	+	+	+	+	+	•	•	•
	+	+	—	+	+	+	+	+	+	+	+	•	•
8 Survived but killed on the 14th day	+	+	+	—	+	+	+	+	+	+	+	40	40
	+	+	+	—	+	+	+	+	+	+	+	20	40
	+	+	—	—	—	+	+	—	—	—	—	80	80
	+	+	—	—	+	—	—	—	—	—	—	40	40
	—	—	+	—	+	+	+	+	—	—	—	80	80
	—	+	—	—	—	—	—	—	—	—	—	40	20
	—	—	—	—	—	—	+	—	—	+	—	80	40

Remarks.

H.B.: Heart blood, Li: Liver, S: Spleen, M.L.: Mesenteric lymph node, Lu: Lung, K: Kidney, C: Caecal content, I: Ileal content.

*: Enlargement and white foci.

**: Widal test.

•: Not examined.

broth. One ml of each inoculum was administered to a mouse by a stomach catheter. Isolation of *Salmonella* was performed by a qualitative culture method on the feces (3-4 g) collected daily from each cage, and on the specimens (see Table 2) obtained from died or sacrificed mice. Enrichment culture of *Salmonella* in the fecal

specimen or intestinal content was done with Hajna's tetrathionate broth (Eiken) and isolation with deoxycholate-hydrogen sulfide-lactose (DHL) agar plate (Eiken) as described previously [5]. The specimens other than those mentioned above were directly smeared on DHL and 5% horse blood agar plate and incubated at 37°C

Table 3. Results on mice given fresh isolate of *S. typhimurium* with or without *Salmonella*-free dog feces

Inocula	Dose* (cells)	No. of mice	Excretion of organism		At sacrifice**		
			1st day	2nd-14th day	Gross lesion	Recovery of organism	Antibody response
Fresh isolate	5	5	—	—	—	—	—
	55	5	—	—	—	—	—
	555	5	+	—	—	—	—
Fresh isolate	6	5	—	—	—	—	—
mixed with Sal-	60	5	—	—	—	—	—
monella-free	600	5	—	—	—	—	—
dog feces	6,000	5	+	—	—	—	—

Remarks.

*: The number of *S. typhimurium* administered per mouse.

**: All mice survived and were sacrificed on the 14th day postadministration.

for 24 hr. Serum agglutinin-titer was also examined by serial dilution method on mice sacrificed at the 14th day. The procedure for agglutination test was exactly the same as reported previously [6].

Fifteen mice given "the organisms in feces" began to excrete *S. typhimurium* 2 or 4 days after administration, and the excretion lasted for almost whole the period of observation (Table 1), though negative results were shown on some days. Seven (46.6%) of 15 mice died within 7 to 12 days. The organism was recovered from all the specimens of the died mice (Table 2). All the died mice showed gross lesions such as enlargement of the liver and mesenteric lymph node with small, irregular and white foci (0.1-3 mm). Five of them also had the lesions in the spleen. Of 8 survived mice, 5 cases showed the gross lesions and antibody with recovery of the organism, and 2 cases had the antibody level with the gross lesions or recovery of the organism. The remaining one showed negative results in all the examinations. On the contrary, in the mice given "fresh isolate" or "fresh isolate mixed with feces", excretion of the organism in the feces was found only on the 1st day after

administration in the mice received the highest dose, 555 or 6,000 cells (Table 3). None of the mice were found to be positive pathologically, bacteriologically and serologically at the sacrifice regardless of inoculating doses. Five control mice given *Salmonella*-free dog feces showed negative results in all the examinations.

As found in the experiment with dogs, *S. typhimurium* harbored in the feces of canine carrier showed higher pathogenicity against mice than the fresh isolate of the serovar. Almost all the mice were infected with *S. typhimurium* and a half of them died of sepsis when they were given orally a small amount of "the organisms in feces", while none of the mice given various doses of "fresh isolate" or "fresh isolate mixed with feces" acquired the infection. In the preliminary experiment by the authors, the mice inoculated intraperitoneally with broth cultures of strains of *S. typhimurium* derived from canine carrier died even in the cases with 10 to 100 cells of the organism. Experimental murine salmonellosis with *S. typhimurium* or *S. enteritidis* by Hobson [1, 2], Meynell [3], Miller and Bohnhoff [4] and Ushiba et al. [7] also showed that normal mice required a great

number of the organisms to initiate infection in case of oral administration, while the mice became infected easily by intraperitoneal inoculation with a small number of the organisms. In another experiment by the authors (unpublished data), a group of mice given orally a small amount (184 cells/mouse) of *S. blockley* harbored in the feces of canine carrier shed the organism for 13 days. At the sacrifice on the 14th day, 2 of the 5 mice were found to be infected with the organism and the other one mouse harbored the organism in the caecal and ileal contents without producing gross lesions and antibody. On the other hand, none of the mice given rather large doses (248–24,800 cells/mouse) of the fresh isolate of *S. blockley* mixed with Salmonella-free dog feces were infected with the organism, except for that a mouse given 24,800 cells. Although there is no available

data to explain the high virulence of “the organisms in feces” in comparing with that of the isolated strains, it seems likely that the factor concerning the virulence exists in the organisms themselves, and it is lost easily by culturing with artificial media, especially with the selective media for Salmonella.

References

- [1] Hobson, D. (1957). *J. Path. Bact.*, **73**, 399–410.
- [2] Hobson, D. (1957). *J. Hyg.*, **55**, 322–333.
- [3] Meyell, G. G. (1955). *Proc. roy. Soc. Med.*, **48**, 916–918.
- [4] Miller, C. P., and Bohnhoff, M. (1962). *J. infect. Dis.*, **111**, 107–116.
- [5] Tanaka, Y., Katsube, Y., and Imaizumi, K. (1976). *Jap. J. vet. Sci.*, **38**, 215–224.
- [6] Tanaka, Y., Katsube, Y., and Imaizumi, K. (1976). *Jap. J. vet. Sci.*, **38**, 569–578.
- [7] Ushiba, D., Saito, K., and Nakano, M. (1962). *Jap. J. exp. Med.*, **32**, 519–529.