NOTES

DYSENTERY PRODUCED BY ORAL ADMINISTRATION OF THE COLONIC CONTENTS OF CYNOMOLGUS MONKEYS INFECTED WITH *SHIGELLA FLEXNERI* 2a

Our previous studies (Honjo et al., 1964, 1967, 1968 ; Ogawa et al., 1964, 1967 ; Takasaka et al., 1967) have shown that clinically pronounced dysentery was developed in about 60 per cent of cynomolgus monkeys orally administered with 10^9 - 10^{10} of a freshly isolated *Shigella flexneri* 2a. However, it seems unlikely that such a large amount as 10^9 - 10^{10} bacilli is ingested at one time to produce clinical manifestation of naturally occurring shigellosis in monkeys. Actually, the oral administration with 10^6 bacilli could not evoke clinically detectable signs of shigellosis (Takasaka et al., 1967). On the other hand, relatively small doses such as 10^5 bacilli directly injected into the cecal lumen could produce dysentery (Takasaka et al., 1968). Recently, Honjo et al. (1969) have demonstrated that the ascorbic acid deficient monkey has, more or less, a lowered resistance, showing the development of dysentery with a relatively small doses of *S. flexneri* 2a in an oral experimental infection system. To ascertain more relevantly the mode of infection in naturally occurring bacillary dysentery, we have conducted the present oral infection experiment under such a condition that the experimentally administered material may have some characteristics more similar to the naturally infecting agent than the cultivated *Shigella* used in the previous experiments.

Cynomolgus monkeys (*Macaca irus*) from the Philippines and Cambodia weighing from 1.8 to 3.5 kg were used. They were conditioned at our animal room for at least 5 weeks or more prior to use. During the conditioning period, stool specimens of each monkey were tested for the presence of *Shigella* bacilli 2 to 8 times with negative results, except that *S. dysenteriae* 2 and *S. sonnei* were isolated from 3 monkeys 2 days before or on the day of the administration.

Virulent *S. flexneri* 2a strain 5503 (Ogawa et al., 1966) was used in this experiment.

As the 1st step of this experiment, 10^9 - 10^{10} bacilli grown on nutrient agar plates at 37°C for 18 hr were administered into the stomach of 12 starved monkeys by the same method as described previously (Honjo et al., 1964), resulting in the development of pronounced clinical dysentery in 8 of the 12 infected monkeys within 2 days after the challenge. Then, 5 of 8 dysenteric animals were sacrificed 2 days after the challenge when they excreted typical dysenteric diarrhea and the contents of the colon were collected and homogenized with sterile sand glass in a sterile mortar. Various amounts of the homogenized dysenteric contents were suspended in sterile saline or distilled water and the volume of suspension was adjusted to 10 ml.

As the 2nd step, these suspensions were immediately administered by the

Table 1. The amount of dysenteric contents of the colon administered,

Group	Amount of dysenteric content of the colon given	No. of viable <i>S. flexneri</i> 2a in dysenteric content of the colon given	No. of animals used
1	1000 mg	$10^{7.4}-10^{9.0}$	12
2	1-10 mg	$10^{4.8}-10^{7.0}$	16
3	0.1 mg	$10^{3.2}-10^{5.0}$	16
4	0.001 mg	ca. 10^1	4

* Titers are expressed by the logarithm to the base of 2 of the endpoint serum dilution.

In the calculation of averages, titers lower than 2^2 were scored as 2^1 .

oral route into the stomach of 48 monkeys, that had been starved for about 24 hr prior to the administration. As shown in Table 1, group 1 was administered with 1000 mg of the dysenteric contents having about 10^7 - 10^9 viable *S. flexneri* 2a, while groups 2, 3 and 4 were administered with 1-10 mg (viable *S. flexneri* 2a: about 10^4 - 10^7), 0.1 mg (viable *S. flexneri* 2a: about 10^3 - 10^5) and 0.001 mg (viable *S. flexneri* 2a: about 10^1), respectively. The challenged monkeys were observed for whether or not dysentery symptoms developed during the experimental period of 21 days.

Bacteriological procedures for isolation and identification of *Shigella* in stool specimens were the same as described previously (Honjo et al., 1964).

Serum agglutination titers against *S. flexneri* 2a were determined by the bacterial agglutination test (Honjo et al., 1967).

Microscopic observation of the lesion and detection of *Shigella* in gastrointestinal tissue were carried out by the routine histological method and by the fluorescent antibody technique described previously (Ogawa et al., 1964).

As shown in Table 1, 3 of 12 monkeys in group 1 began to excrete a large quantity of bloody mucous stool 2, 3 and 4 days after the administration (3 days on the average in the 3 cases). In one dysenteric case that began to excrete dysenteric diarrhea after 3 days, diarrhea continued for 3 days until the monkey died. Four of 16 monkeys in group 2 developed dysentery after 4 or 5 days (4.3 days on the average). In 3 of 16 monkeys in group 3, bloody mucous diarrhea appeared after 4 or 9 days (7.3 days on the average). One of these 3 dysentery-infected monkeys suffered from especially serious diarrhea and died 2 days after the onset of clear clinical symptoms. On the other hand, all of the 4 monkeys in group 4 administered with 0.001 mg of dysenteric contents were free of dysentery symptoms during the observation period of 21 days.

In all the dysenteric cases of groups 1, 2 and 3, the number of *S. flexneri* 2a per gram of the stool ranged from 10^7 to 10^9 , showing almost the same tendency as the stool of monkeys infected orally with a freshly isolated *S. flexneri* 2a strain in the previous studies (Honjo et al., 1964; Takasaka et al., 1967). Upon bacteriological examinations of the rectal swab, 8 of 11 monkeys (72.7 %) in group 1, 9 of 16 monkeys (56.3 %) in group 2 and 3 of 15 monkeys (20 %) in group 3

the number of dysenteric cases produced and serum agglutinin titers

No. of dysenteric cases	Average incubation period (days)	Average serum agglutinin titer*	
		Before challenge	2 weeks postchallenge
3	3	3.4	5.3
4	4.3	3.5	5.9
3	7.3	3.4	4.1
0		2.7	3.3

were found positive for *S. flexneri* 2a on the 8th day after the administration with diarrheal materials, and, thereafter, 2 of 10 monkeys (20 %) in group 1, 3 of 13 monkeys (23.1 %) in group 2 and 3 of 14 monkeys (21.4 %) in group 3 gave positive results for *S. flexneri* 2a isolation from stools on the 15th day after the administration, while from all of the 4 monkeys in group 4 no *Shigella* bacilli

Table 2. Summary of clinical, pathological and bacteriological findings in monkeys autopsied

Group	Animal No.	Appearance of clinical sign* (days after the challenge)	Fate** (days after the challenge)	Large-intestinal lesions		Isolation of <i>S. flexneri</i> 2a from the gastrointestinal tract****			
				Inten- sity	Penetra- tion of <i>S. flexneri</i> 2a***	Lower small intestine	Cecum	Colon	Rectum
1	3	P (4)	S (8)	Slight	±	+		—	+
	4	P (3)	D (5)	Severe	‡	+		9.6	9.3
2	2	P (4)	S (8)	Moderate	+	—		+	+
	6 ⁽¹⁾	N	D (9)	Moderate	—	—	—	—	—
	16	P (5)	S (7)	Severe	‡		7.8		8.3
3	2	P (4)	D (5)	Severe	‡	+		9.6	9.3
	8	N	S (21)	None	—	—	—	—	—
	9	P (9)	S (10)	Severe	‡	+		6.7	7.3

* P : Positive (bloody mucous stool), N: Negative.

** D : Died, S: Sacrificed.

*** ‡: Widely penetrated into the epithelial lining and lamina propria,
+ : Penetrated into the surface epithelium, not into the deeper crypt,
± : A few epithelial cells harbored *S. flexneri* 2a,
— : No penetration.

**** Number of *S. flexneri* 2a isolated per gram of intestinal content is expressed as logarithm to the base of 10.

+ : *S. flexneri* 2a positive, — : *S. flexneri* 2a negative.

- (1) The monkey died of muddy mucous diarrhea 9 days after the administration, a moderate catarrhalic colitis was observed in the large intestine from where *S. dysenteriae* 2 was isolated in number of $10^{7.9}$.

were isolated throughout the observation period of 21 days.

Serum agglutinin titers in almost all monkeys of groups 1 and 2 showed a trend of two- or fourfold rise 1-2 weeks after the administration, but the titer rises of the other 2 groups were only twofold or less after 2 weeks (Table 1). These trends of antibody titer rise were noted regardless of the presence or absence of dysentery symptoms.

Table 2 summarizes gross and histological findings, including both the detection of fluorescing bacilli and the isolations of bacilli from various parts of the gastrointestinal tract of the animals autopsied 5-21 days after the administration. In all of the dysenteric cases, no pathologic change was observed in the stomach and the small intestine, while various degree of inflammatory reaction and bacterial penetration were observed in the large intestine without exception. For example, in a dysenteric case No. 3 of group 1 autopsied 8 days after the administration when the stool property returned back to normal, only microfoci containing fluorescing bacilli in epithelial cells of the limited areas were observed in the rectum, and also inflammatory response was limited to the lamina propria just under the parasitized epithelia.

Four monkeys (No. 4 of group 1, No. 16 of group 2, and Nos. 2, 9 of group 3) autopsied 1-2 days after the onset of dysentery showed severe changes, that is, the hyperemia, hemorrhage, oedema, and erosion of the mucous membrane were observed on the extensive areas of the large intestine, and *S. flexneri* 2a penetrated into the deeper crypt and the lamina propria. These findings were almost the same as those in the infection of a freshly isolated *S. flexneri* 2a of previous studies (Ogawa et al., 1964, 1966). In No. 6 of group 2 that died of muddy mucous diarrhea after 9 days, a moderate catarrhalic colitis was observed in the large intestine from where *S. dysenteriae* 2 was isolated, although no penetration of *S. flexneri* 2a into the epithelium of the catarrhalic areas was observed as well as negative cultures of *S. flexneri* 2a from various parts of the gastrointestinal tract were obtained. It can be considered, therefore, that the development of a mild form of dysentery was caused by naturally infected *S. dysenteriae* 2.

In short, the present study demonstrates that, when a relatively small amount of fresh dysenteric contents of the colon of monkeys was orally administered, dysentery with the same clinical and pathological findings as those in the oral challenge with a dose of freshly isolated *S. flexneri* 2a as large as 10^9 - 10^{10} could be produced.

On the basis of the present results, we consider that the following three possibilities must be elucidated in further studies with regard to the virulence of *Shigella* bacilli: (1) the virulence of *Shigella* bacilli being present in fresh dysenteric contents of the colon is essentially higher for monkeys than that of *Shigella* bacilli freshly isolated from dysenteric contents on a culture medium, (2) some substances in fresh dysenteric contents enhance the infectivity of *Shigella* bacilli, and (3) some substances in fresh dysenteric contents inhibit the decline of the virulence.

Recently, Nakamura et al. (1966) and Nakamura (1967) showed that a loss of virulence of *Shigella* bacilli during cultivation on culture media resulted from reduction in the ratio of the number of virulent bacilli to the total bacilli grown, by employing such a test system as keratoconjunctival infection in guinea pigs

and infection of cell cultures with *Shigella*. Thus, this fact also seems worthwhile to be taken into consideration when our present results are discussed, regarding the virulence of *Shigella*, that is, the dysenteric contents administered in the present experiment may have contained virulent bacilli exceeding in number the suspension of freshly isolated bacilli used in our previous experiment.

Furthermore, the present experiment may suggest that the ingestion of at least 10^4 – 10^5 (about 0.1–10 mg in terms of the amount of fresh dysenteric stool) *Shigella* bacilli is necessary for the establishment of infection in naturally occurring dysentery of monkeys.

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