Experimental Study on the Parenteral Use of Antibiotics for the Treatment of Bacterial Diarrheal Diseases

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Abstract: The movement of parenterally given antibiotics to the diarrheal stool was studied by using two kinds of adult rabbit model for experimental cholera (T-model and Intestinal loop model). Four antibiotics (cefotiam=CTM, 20mg/kg; gentamicin=GM, 3 mg/kg; minocycline=MINO, 10mg/kg; rifampicin=RFP, 5mg/kg) were administered with intravenous shot to the animals infected with cholera. All drugs appeared in the stool soon after medication. The concentration of the drugs in the stool was highest in GM, and lowest in CTM. RFP was detected from the stool for more than 24 hours after the single dose, but CTM was not detected after 8 hours. The pathway of GM from the blood vessels to the intestinal lumen was almost totally through the intestinal mucosa (mucosal pathway), while that of RFP was mainly through the bile system (hepatic pathway). Mucosal pathway was predominant in the movement of MINO. Hepatic pathway was predominant in CTM, although the movement of this drug was not much directed to the intestine. Different from antibacterial activity *in vitro*, GM did not work against the pathogen at all *in vivo*. *Vibrio cholerae* in the intestine kept on proliferating and producing cholera toxin under the presence of GM in high concentration. RFP showed an excellent bactericidal effect as well as *in vitro*, but a problem of naturally resistant strain with the frequency of 1 to 10^6-10^7 remained. The necessity of abundant defecation to eliminate the pathogen was suggested in the case with bacteriostatic antibiotics such as MINO. The benefit of parenteral use of antibiotics for the treatment of bacterial diarrheal diseases was discussed.

Key Words: Experimental cholera, T-model, Antibiotics.

INTRODUCTION

Acute diarrheal diseases are recognized as a major cause of mortality and morbidity of young children in the tropical area. In many countries, it is said that over one-third of the beds in childrens hospital or wards are occupied by the patients suffering from diarrhea (Wkly. Epidem. Rec. WHO, 1982, No. 22). For the treatment of ba-

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cterial diarrheal diseases, the use of antibiotics is one of the beneficial maneuvers except some cases with salmonella (Aserkoff and Bennett, 1969). But in any case, antibiotic therapy should be given under the knowledge of metabolism and distribution of the drugs in the body. It is required that the given drugs get effectively into the infection site. When a certain antibiotics is coming up to the market, detail studies on the toxicity, the metabolism, the clinical efficacy etc. are carried out. The drug distribution in the body (blood, urine, bile, cerebrospinal fluid, ascites, and even in the tissue of organs) is also studied. But in general, the drug distribution in the stool is almost not determined. This is unreasonable, because there are many infectious diseses in the intestine which need antibiotic therapy. Iwanaga *et al.*(1970) reported on the concentration of chloramphenicol (CP) in cholera stool. And they described that when CP was given intravenously, it soon appeared in the stool with high concentration, and the vibrio in the stool disappearred within 6 hours after medication. But when CP was given by mouth, the drug was almost not detected in the stool and the number of viable vibrio in the stool did not decrease. It was clarified later on that orally given CP was inactivated with glucuronidation (data not published). In the following year, Nakatomi (1971) confirmed the movement of CP in cholera patients. Except these two papers, the concentration of antibiotics in the stool after parenteral administration has not been described so far. In this paper, the movement of parenterally given antibiotics in the animals with experimental diarrhea, especially the concentration in the stool, is described.

MATERIALS AND METHODS

Animals: Rabbits of either sexes (Japan White), weighing 2 to 3 kg were used. All rabbits were apparently healthy, and were starved for 24 hours but water ad lib. prior to challenge.

Models for experimental diarrhea: 1. Ileal tube method (T-model). This model procedure was previously described by Iwanaga and Ichinose in this volume. 2. Intestinal loop model. Four, five, or six intestinal loops originated by De and Chatterjee (1953) were made. The length of the loops was various from 10 to 30 cm, and about the same length of uninoculated portions were taken between the loops. Inoculation was done with about 10^6 of freshly cultured pathogen in 0.1 ml of heart infusion broth (Eiken) per loop.

Pathogen: *Vibrio cholerae* O1 isolated from human stool in Kenya in 1980 (our laboratory No. A-66) was used throughout this study. Since the isolation, the strain has been maintained in a butt of nutrient agar (Eiken) with subcultures every 3 to 4 months at room temperature.

Antibiotics: Cefotiam (Takeda), gentamicin (Shionogi), minocycline (Lederle Japan), and rifampicin (Dai-ichi) were used with the dosages of 20, 3, 10, 5 mg/kg, re-

spectively. The minimum inhibitory concentration (MIC) of these drugs against the pathogen used (strain A-66) *in vitro* with agar plate dilution method were 0.39 mcg/ml of minocycline and 0.78 mcg/ml of the other three drugs.

Medication and treatment for the infected animals: In the animals of T-model, continuous intravenous fluid infusion therapy with lactated Ringer's solution containning 5% glucose was started when the collapsed blood vessels in the rabbit ears were observed. The antibiotics were given with intravenous shot after the development of diarrhea was confirmed by sucking the diarrheal fluid through the tube. In the animals of loop model, the antibiotics were given with intravenous shot in 8 hours after challenge. The animals were sacrificed in 3 hours thereafter. Fluid therapy was not given

Determination of antibiotics: The concentration of antibiotics in the stool (sucked fluid through the tube, and accumulated fluid in the loops), blood, urine and bile in the bladders were determined by bioassay technique with thin layered cup method using heart infusion agar (Eiken) plates. As the indicator organism, *Bacillus subtilis* strain ATCC 6633 was used.

Titlation of cholera toxin: Cholera toxin (CT) in the stool samples were titlated with reversed passive latex agglutination method. The preparations of purified CT (choletox; chem-sero therapeutic Institute) and antiCT sensitized latex particles (V-ET RPLA; Denka seiken Co.) were commercially obtained. Stool samples were centrifuged and filtered through 0.45 μ milipore filter, and the filtlates were titlated for CT.

RESULTS

T-model: In the case with cefotiam (CTM), the amount of sucked stool during the first 5 hours after medication was 138 ml. The drug concentration in the stool reached a maximum level of 1.6 mcg/ml in 2 hours after medication with 20 mg/kg. The number of live vibrio in the stool was steadily going down from the level of 10^9/ml to 10^5/ml, until the drug in the stool disappeared. After the disappearance of the drug in the stool, the number of live vibrio went up to the initial level (Fig. 1). In the case with gentamicin (GM), the amount of sucked stool during the first 5 hours was 102 ml. The drug appeared in the stool very soon after medication, and it maintained high concentration for a long time. The peak concentration was seen in 8.5 hours after medication, but the slopes before and after were not steep. Nevertheless, the number of live vibrio in the stool did not decrease at all. In this case, the second dose was given at the 16th hour from the first dose, but the antibacterial effect was not observed. CT concentration in the stool kept high level during the time process in spite of large amount of of the stool was extracted (Fig. 2). In the case with minocycline (MINO), the amount of sucked stool during the first 5 hours after medication was 39 ml. The concentration of the drug reached a maximum of 2.7 mcg/ml in 1 hour and a half after the medication with 10 mg/kg. In 8 hours, only a trace of the

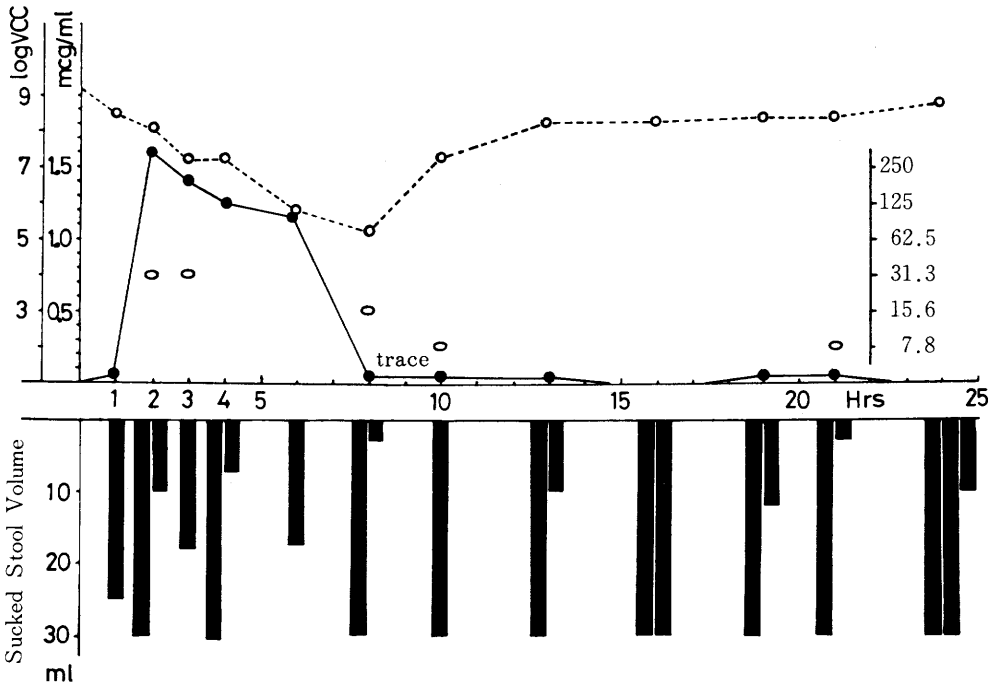


Fig. 1. ●——● CTM concentration in the stool (mcg/ml)
o- - - -o Number of viable vibrio in the stool (log/ml)
O CT concentration in the stool (ng/ml)

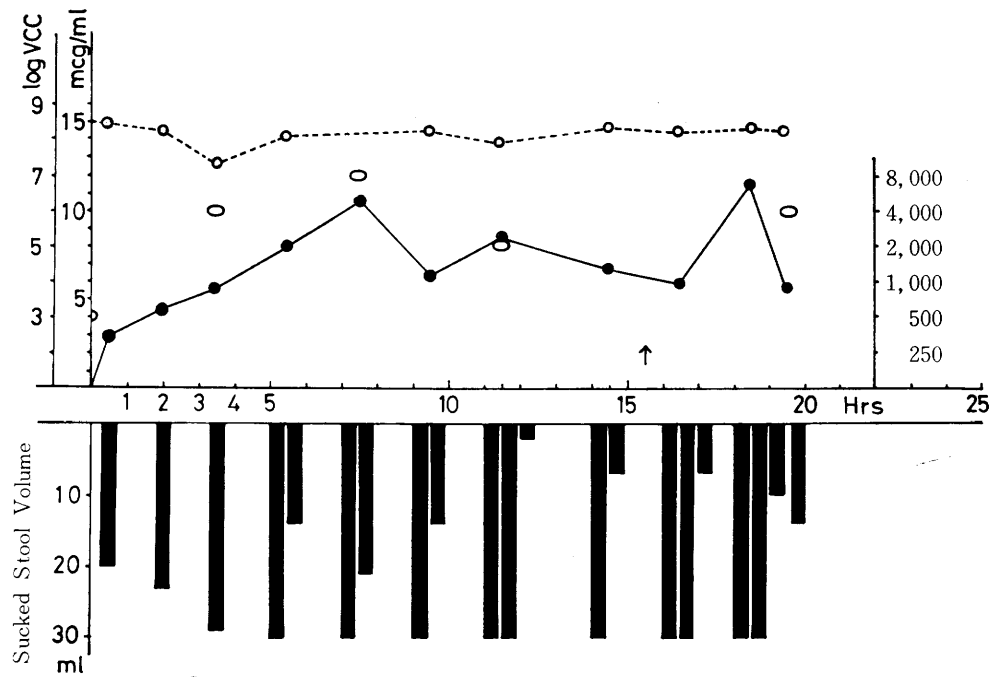


Fig. 2. ●——● GM concentration in the stool (mcg/ml)
↑ second dose (GM 3mg/kg)

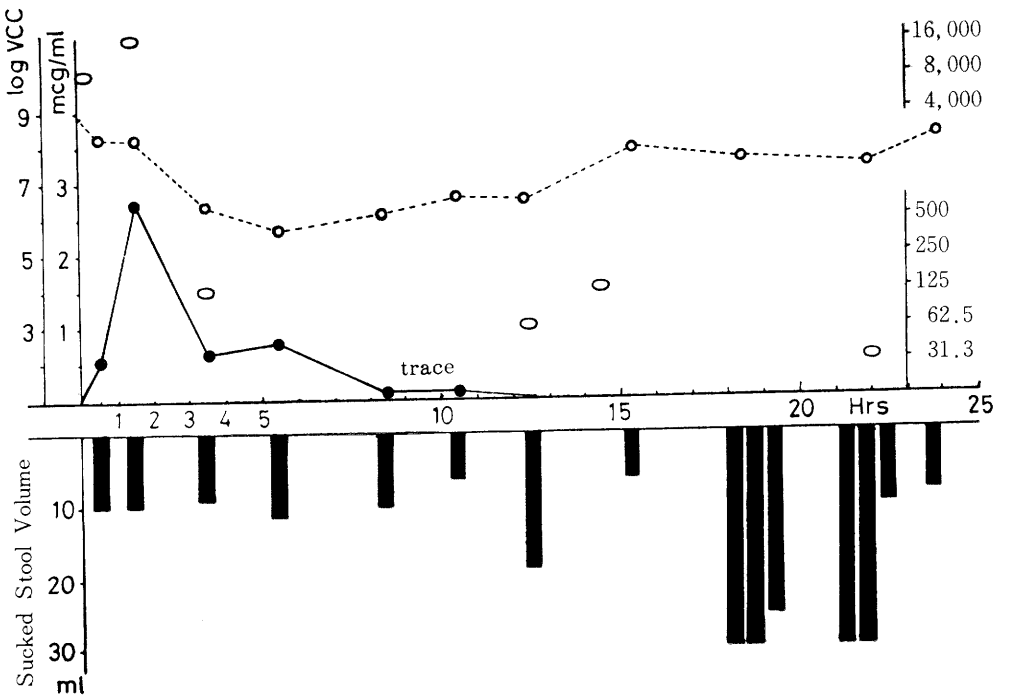


Fig. 3. MINO concentration in the stool (mcg/ml)
Number of viable vibrio in the stool (log/ml)
CT concentration in the stool (ng/ml)

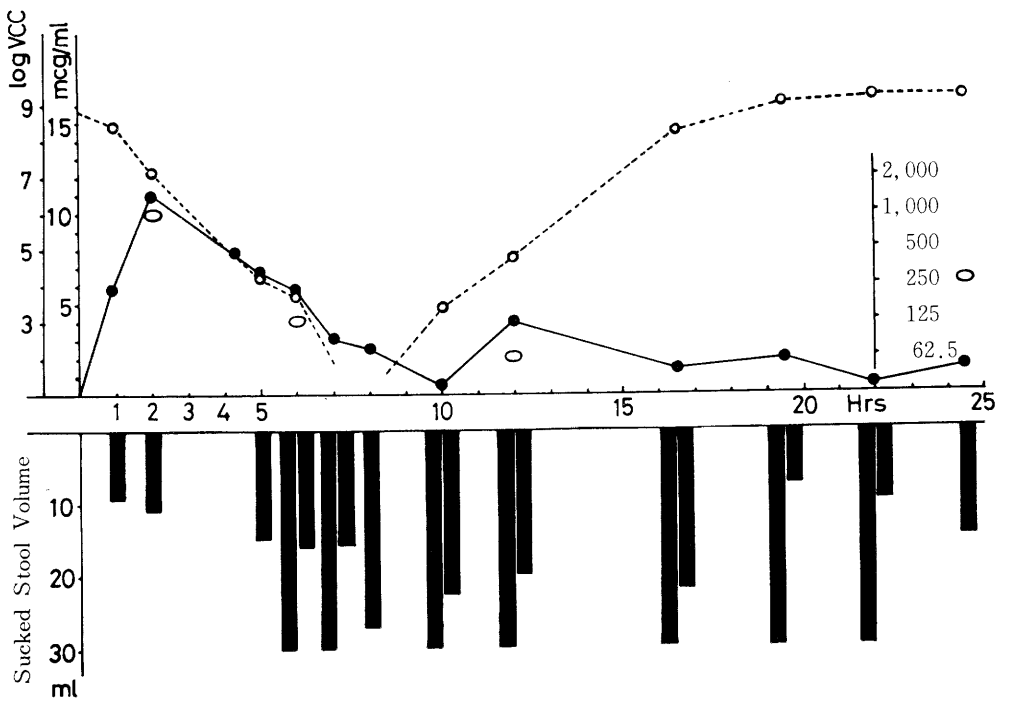
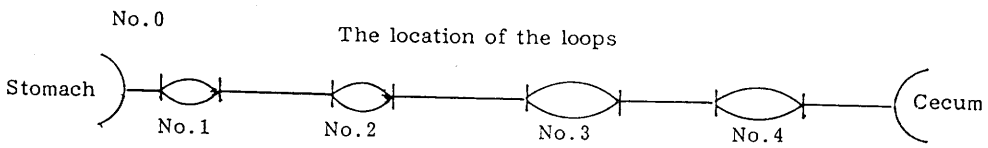


Fig. 4. RFP concentration in the stool (mcg/ml)

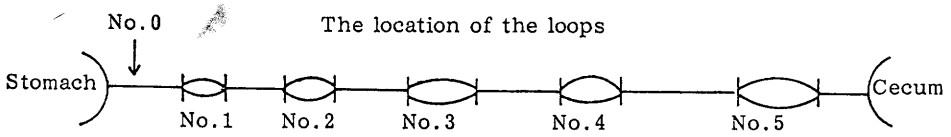
Table 1. The loop test for Cefotiam (20mg/kg iv)



Loop No.	Length (cm)	fluid (ml)	FAR	Cefotiam mcg/ml	Cefotiam total (mcg)	VCC/ml
1	20	34	1.700	0.49	16.7	5×10^8
2	20	1.3	0.065	1.25	1.6	2×10^9
3	37	9	0.243	0.93	8.4	5×10^8
4	32	20	0.625	0.74	14.8	2×10^8
No.0	12	++		1.55		(-)

CTM concentrations in: Blood 0.5, Urine 1,200, Bile 26. (mcg/ml)

Table 2. The loop test for Gentamicin (3mg/kg iv)



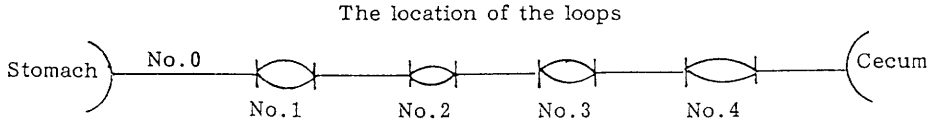
Loop No.	Length (cm)	Fluid (ml)	FAR	Gentamicin mcg/ml	Gentamicin total (mcg)	VCC/ml
1	13	30	2.308	12.0	360.0	1.2×10^8
2	15	5	0.333	13.0	65.0	2.2×10^8
3	21	4	0.190	15.0	60.0	3.2×10^8
4	19	5	0.268	11.7	58.5	3.0×10^8
5	27	14	0.518	7.6	106.4	2.0×10^8
No.0	24	++		1.7		(-)

GM concentrations in: Blood 9.3, Urine 590, Bile 8.8. (mcg/ml)

drug was detected. Following the maximum level, the number of vibrio in the stool began to decrease. It decreased from 10^9/ml to 10^6/ml. But in accordance with the disappearance of the drug, the number of vibrio increased again and kept the level of 10^8/ml thereafter (Fig. 3). In the case with rifampicin (RFP), the amount of sucked stool during the first 5 hours was 36 ml. The concentration of the drug reached a maximum of 11.0 mcg/ml in 2 hours. It decreased steadily thereafter and once it almost disappeared from the stool in 10 hours. But the drug appeared again in the stool. And the concentration was increasing and decreasing repeatedly with lowering the level.

Table 3. The loop test for Minocycline (10mg/kg iv)

The location of the loops

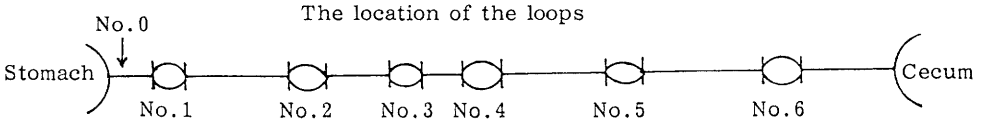


Loop No.	Length (cm)	Fluid (ml)	FAR	Minocycline		VCC/ml
				mcg/ml	total (mcg)	
1	20	5	0.250	3.8	19.0	1.2×10^7
2	16	14	0.875	3.2	44.8	2.5×10^7
3	17	20	1.176	2.2	44.0	9.0×10^6
4	24	30	1.250	1.6	48.0	2.0×10^7
No.0	48	++		1.2		(-)

MINO concentrations in: Blood 17, Urine 48, Bile 34. (mcg/ml)

Table 4. The loop test for Rifampicin (5mg/kg iv)

The location of the loops



Loop No.	Length (cm)	Fluid (ml)	FAR	Rifampicin		VCC/ml
				mcg/ml	total (mcg)	
1	10	15	1.500	2.2	33	3.7×10^4
2	9	10	1.111	1.3	13	$<10^2$
3	10	9	0.900	2.2	19.8	〃
4	13	2.5	0.192	1.6	4	〃
5	8	7	0.875	1.1	7.7	〃
6	12	7	0.583	1.7	11.9	〃
No.0	15	++	———	2.3	———	(-)

RFP concentrations in: Blood 1.3, Urine 9.6, Bile 39. (mcg/ml)

In the beginning, the volume of extracted stool was small, nevertheless, the number of vibrio decreased rapidly to undetectable level (less than 10^2/ml). But after a few hours observation of vibrio free stool, the organism appeared again and increased their number without any suppression by RFP still present in the stool. This reappeared vibrio was highly resistant to RFP (MIC of RFP$>$100 mcg/ml).

Loop model: The results were summarized in Table 1 to 4 with an illustration of

the anatomical location of the loops in each case. No.0 portion is the duodenum between the stomach and the first loop. Greenish, rather mucous fluid was accumulated there because of ileus condition. Of cause, vibrio was not inoculated there and not detected when the animals were sacrificed. The drugs in this portion was regarded as that came with bile secretion. The concentration of CTM in the loops was lowest of all four drugs tested, although large amount was administered. The loop No. 2 which showed the concentration of 1.3 mcg/ml was almost negative in the fluid accumulation (FA ratio=0.065). Therefore, the data may not be reliable. The concentration in No. 0 portion was definitely higher than that in the loops (Tab.1). When GM was given, the concentration in the loops got very high, but that in No.0 portion was much lower than that in the loops. Even in the gall bladder bile, the concentration was lower than that in the loops. The vibrios in the loops were not suppressed at all in this high concentration of GM (Tab.2). In the case with MINO, the concentration in No.0 portion was also lower than that in the loops. The drug retained in the blood with high level (Tab.3). The concentration of RFP in the loops were relatively low, comparing with that seen in the T-model. But almost all vibrio in the loops were killed with 1 to 2 mcg/ml of this drug. The concentration in the bile was much higher than that in the urine (Tab.4).

DISCUSSION

This study proved that parenterally given antibiotics appeared in the intestinal lumen, and it gave a warranty to the treatment with parenteral use of antibiotics for bacterial diarrheal diseases. And the two pathways of the drugs from the blood vessels to the intestinal lumen were clarified. Getting into the intestinal lumen, GM almost totally depends on the pathway through the intestinal mucosa (mucosal pathway), and RFP mainly depends on the pathway through the bile system (hepatic pathway). MINO is a bacteriostatic antibiotics, therefore, the decrease of viable vibrio in the stool depends on the amount of defecation. In this presented case, the volume of sucked stool was not large, but 99.9 % of vibrio was once eliminated with one dose of MINO. Of cause, this degree of elimination is not satisfactory from bacteriological point of view. Much larger amount of defecation is necessary for the complete elimination of vibrio from the stool, as seen in the human cases with chloramphenicol which was reported by Iwanaga *et al.* (1970). Or second dose of MINO should be given. In the intestinal loop model, MINO passed through the mucosa and appeared in the loops. Although the concentration in No.0 portion was lower than that in the infected loops, greenish fluid accumulated in that portion and high concentration in the bile suggested that MINO in the stool of the T-model partially came from the hepatic pathway. The different concentration in each loop may depend on the time of medication and fluid accumulating stage, most likely same as the other three drugs. If the fluid was already

accumulated to some extent at the time of medication, the drug would be diluted by the fluid already there.

GM has a bactericidal activity against the used pathogen (strain A−66) in the concentration of 4MIC (MIC=0.78 mcg/ml, 4MIC= 3.13 mcg/ml) as far as the study *in vitro* is concerned. GM concentration in the stool was very high in both models, and the high level was maintained for a long time. Besides, a large amount of stool was intermittently sucked. Nevertheless, the number of vibrio and amount of CT in the stool kept high level throughout the experiment. It suggested that the vibrio kept on proliferating and producing CT even under the presence of GMwith high concentration. GM did not work at all against the pathogen *Vibrio cholerae*. It is known that antibacterial activity of GM is very weakened under the acid condition (Mori and Iwanaga, 1980). But pH of the stool in this case ranged from 7.15 to 8.20 during the first 20 hours of the disease. GM concentration in the bile and No.0 portion was very low, which suggested that almost all amount of GM in the stool had come through the mucosal pathway.

CTM is a bactericidal antibiotics of beta-lactams. The routine clinical use of amount but relatively large dose comparing with the other drugs were given, but the movement of CTM was not much directed to the intestine. Especially the drug was poorly permeable through the intestinal mucosa, and did not show the effective elimition of vibrio in the stool.

When RFP was given intravenously, although the preparation of RFP for the parenteral use is not available in clinic, it appeared in the stool with high concentration. RFP maintained the significant level in the stool for a long time. RFP has a strong bactericidal activity against *Vibrio cholerae*, but unfortunately, one per 10^6-10^7 of the vibrio was naturally resistant to RFP (data not shown). It was definite that RFP in the stool after intravenous administration was mainly passed through the hepatic pathway because of low concentration in the loops and high concentration in the bile and No.0 portion. If RFP is absorbed from the intestine with cholera, it must be secreted again into the intestine through the bile. It will repeat absorption and secretion running on this entero-hepatic circulation. This idea is the reason why we tried RFP for the treatment of cholera in this experiment. We expected to succeed "one dose therapy with RFP" for cholera. But when RFP was given by mouth, the stool with high concentration of the drug was seen only for a short duration (Fig.5). When the animal was sacrificed, the RFP concentration in the blood, the urine, and the bile were very low, which suggested poor absorption of RFP in cholera case.

It is known that glucose raise the absorptive ability of the intestine with cholera (Fisher and Parsons, 1953; Phillips, 1964). And this mechanism has been applied to the oral rehydration therapy (Barua and Burrows, 1974). We did not try to give RFP with glucose by mouth, but anyway, "one dose therapy" has become hopeless because

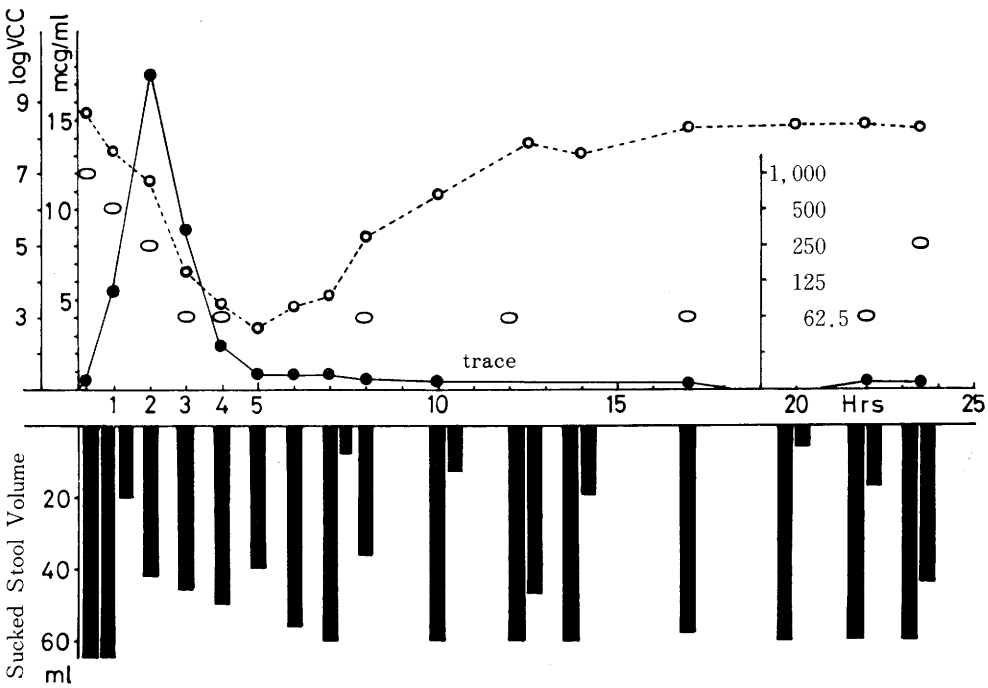


Fig. 5. RFP concentration in the stool after oral administration (mcg/ml)
Number of viable vibrio in the stool (log/ml)
CT concentration in the stool (ng/ml)

of resistant strain.

In the patients with gastroenteritis, the oral administration of the drugs is not always favourable because of nausea and vomiting, or unconsciousness. Besides, these patients may have absorptive disfunction. In the cases with cholera, the antibiotics given by mouth may pass through the intestinal lumen without immersing in the deep crypt already filled with transudative cholera fluid. In the cases with dysentery, the site of the infection (lamina propuria mucosa, colon) is covered with fibrinous pseudomembrane. Exudate movement is directed from the tissue to the lumen. In such condition, not much of the antibiotics in the intestinal lumen can reach the infection site. Even in these cases, parenterally given antibiotics are distributed by the blood stream and diffusely immersed the whole intestinal tissue in the drugs. Then the drugs ooze out to the intestinal lumen with the exudate or transudate. This pattern of the antibiotic movement in the body sounds like ideal. The methods of the use of parenteral antibiotics for bacterial diarrheal diseases should be studied further.

REFERENCES

1) Aserkoff, B. & Bennett, J. V. (1969): Effect of antibiotic therapy in acute salmonellosis on the fecal excretion of salmonellae. New Engl. J. Med., 281, 636−640.
2) Barua, D. & Burrows, W. (1974): CHOLERA p. 253−261, W. B. Saunders Co., Philadelphia, London, Toronto.
3) Fisher, R. B. & Parsons, D. S. (1953): Glucose movement across the wall of rat small intestine. J. Physiol. (Lond.), 119, 210−213.
4) Iwanaga, M., Sogame, S. & Kobari, K. (1970): The investigation of chloramphenicol in treatment of cholera. Trop. Med., 12, 98−112.
5) Mori, K. & Iwanaga, M. (1980): Studies on antibacterial activities of gentamicin. CHEMOTHERAPY, 29, 482−487.
6) Nakatomi, M. (1971): Evaluation of effectiveness of chemotherapy in cholera. Trop. Med., 13, 26−43.
7) Phillips, R. A. (1964): Water and electrolytes losses in cholera. Fed. Proc., 23, 705−712.

細菌性下痢症における抗生剤の非経口的投与について　−ウサギ下痢症における実験的研究−

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我々が最近新しく開発した感染症モデル（ウサギコレラ）を使用して，非経口的に投与された抗生剤の便中への移行とその効果並びに移行経路について検討した．非経口的に投与された各種抗生剤は，急性期コレラにおいて，まもなく便中に出現した．その移行率はゲンタマイシンでは非常に高率であり，セフォチアムでは僅かな移行しかみられなかった．ミノサイクリンは中等度であり，リファンピシンの移行は良好であった．1回投与の後，ミノサイクリンとセフォチアムは8〜10時間で便中から消失したが，ゲンタマイシンとリファンピシンは長時間にわたって便中から検出された．腸管ループテストとの結果と比較することによって，便中のゲンタマイシンはその殆ど全てが腸粘膜を通過してきたものであり，リファンピシンはその大半が肝・胆系を通じて便中に現われるということが判明した．ミノサイクリンでは両経路の差は少ないが粘膜経路をとるものが多く，セフォチアムでは肝・胆経路をとる量の方がやや多かった．便中のゲンタマイシン濃度は，使用したコレラ菌に対する *in vitro* での最小発育阻止濃度を10〜20倍も上まわっていたが，コレラ菌の増殖と毒素産生は全く抑制されていなかった．リファンピシンの殺菌効果は卓越していたが，10^{-6}〜10^{-7}の割合で自然耐性菌が存在し，一旦便中のコレラ菌が消失したかに見えてもまもなく高度耐性菌による増殖がみとめられた．

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