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The Rhesus Monkey as an Experimental Cholera Model[1]

Mr. Syed Iqbalul Hasan; Dr. W. B. Greenough III; Dr. R. S. Gordon, Jr.; and Dr. A. S. Benenson.

Pakistan-SEATO Cholera Research Laboratory, Mohakhali, Dacca 5, East Pakistan.

A series of 97 rhesus monkeys have been prepared and challenged 137 times with *Vibrio cholerae*. The initial studies were directed at duplicating the reports of Mendoza (*1*) and Pottevin and Violle (*2*). Subsequently, malnutrition (including Vitamin C deficiency with clinical scurvy), hypokalemia, and alkalinization were induced prior to challenge. Finally, based on the report by Dutta and Oza (*3*) that absence of pancreatic lipase and phosphorylases might be responsible for susceptibility of the infant rabbit to cholera, the common bile duct and/or pancreatic ducts were interrupted in a series of monkeys prior to challenge. To date, however, no procedures have produced consistent susceptibility to cholera. In most groups a few monkeys did develop a syndrome resembling clinical cholera, with no apparent relation to the particular prechallenge state.

Methods

Rhesus monkeys weighing 1.5–4 kg. were used. They were obtained from dealers who had caught them in the Sunderbans (a jungle region of East Pakistan). Normally they were fed fruit, rice, dhal (a pulse soup), fish and salt. During experiments they were kept in separate cages. Prior to experiments, a rectal swab was cultured for bacterial pathogens; body weight, temperature, and general appearance were noted, and blood was taken. After challenge, rectal swabs were done daily. Liver biopsies were performed on some with the Menghini needle after an iodine-alcohol skin preparation. An autopsy was performed on animals that died; at autopsy the abdomen was opened and the intestines were clamped at different levels before further manipulation. For sacrifice, animals were anesthetized, the clamps were placed at different levels of the intestinal tract, and then the animals were killed by an overdose of ether. Cultures of organs for *V. cholerae* were taken by searing the site for aspiration or excision of small pieces of tissues. The gross appearance of organs was noted and blocks of tissue were placed in 10 percent formalin or Zenker's solution. The bacteriologic methods for vibrio isolations are those in standard use in the laboratory: direct plating on tellurite taurocholate gelatin agar and enrichment through bile peptone broth.

Pretreatment.—Monkeys were pretreated for challenge in the following ways:

(1) They were placed on deficient diets consisting of either vitamin-free casein, or rice and salt alone. After varying intervals on these diets, a challenge of *V. cholerae* was given.

(2) They received 10 percent sodium sulfate by stomach tube sufficient to induce diarrhea. The vibrio challenge was given immediately after diarrhea began.

(3) We used the sodium sulfate preparation which was modified to give enough potassium sulfate to prevent excessive potassium depletion before challenge.

(4) We substituted 3 percent sodium bicarbonate solution for the drinking water for 1–2 days before challenge and continued giving it after challenge.

(5) In monkeys prepared by 1–2 days on 3 percent sodium bicarbonate, 10 percent sodium sulfate was given to the point of diarrhea prior to challenge.

(6) We injected animals with 0.1 mg. DOCA for 3–10 days prior to challenge, to induce potassium depletion.

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(7) We tied or cut the common bile duct or the common bile duct and pancreatic ducts, and gave the vibrio challenge 1–4 weeks after surgery.

Challenge.—Various preparations were used for challenging with *V. cholerae*. In all cases the challenge was given *per os*. The usual procedure was to give by stomach tube 25 ml. of a broth culture of *V. cholerae* grown overnight in 1 percent trypticase, 1 percent sodium chloride broth (T_1N_1); the total vibrio counts ranged between 10^6–10^{11} organisms. Both Inaba and Ogawa serotypes recently isolated from cholera patients were used, as well as strains recovered from monkeys dying of cholera-like disease. Vibrios were also grown in rice and rice water, and were fed either once or repeatedly. Some monkeys received fresh "rice water" stool from acute human cases of cholera.

Results

None of the procedures tried to date have established a method whereby the monkey will predictably develop the clinical or autopsy findings of cholera. An acute diarrheal syndrome has been seen sporadically in most challenge groups, with gross autopsy findings similar to those seen in the autopsy of human cholera (table 1). However, control monkeys pretreated by purgatives also died of "choleralike disease." Infection with vibrios, as evidenced by positive rectal swabs persisting for several days, did occur quite regularly in all groups. Five monkeys who had not been challenged deliberately, but were held in the infected animal room, developed positive cultures.

Vibrios were recovered at autopsy in 39 of 69 monkeys which died or were sacrificed up to 61 days after exposure to *V. cholerae*. Of particular interest are the following bacteriologic observations:

(1) The duodenum and small intestine were frequently found to be more heavily and more frequently positive than the large bowel (table 2). This was more evident among the monkeys which were sacrificed than among those which had been dead for some time before cultures were taken. The small intestine was culturally positive when the rectum and colon were negative for vibrios in 12 monkeys which had had negative rectal swabs for 1–15 days before autopsy.

(2) Culture data are available for both bile and duodenum in 33 monkeys. In 13 of these both were positive. The duodenum alone was positive in 18 instances and only the bile was positive in 2 cases, indicating that the vibrios in the duodenum did not necessarily come from the bile.

(3) The liver was cultured in 20 monkeys; 5 were positive for *V. cholerae*. All of these were obtained within 8 days of challenge. Four of these monkeys had undergone bile duct ligation 1–4 weeks earlier. Most of the monkeys with bacteriologically positive livers died of a choleralike illness. There was no suggestion of a relationship between a positive bile culture and either time after challenge or choleralike illness (table 3).

These points are exemplified by monkey No. 123. On August 18, 1964, a ligature was placed about the common bile duct; evidently, this was not firm, since jaundice did not follow. On August 26, 1.1×10^{10} Inaba organisms in 25 ml. of T_1N_1 were introduced by stomach tube; 46 hours later a liver biopsy was bacteriologically negative,

Table 1.—Result of different challenge procedure on infection with cholera vibrios

Challenge procedure	"Cholera-like" deaths	No "Choleralike" disease	Total challenges
(1) Deficient diet with and without water deprivation	0	19	19
(2) 10 percent sodium sulfate purge	[1] 1	21	22
(3) Sodium and potassium sulfate purge	0	4	4
(4) 3 percent sodium bicarbonate	3	13	16
(5) 3 percent sodium bicarbonate plus 10 percent sodium sulfate	[2] 4	11	15
(6) DOCA	1	10	11
(7) Surgical interruption of bile duct	3	37	40
(8) Accidental infection or multiple challenge	0	8	8
Total	12	123	135

[1] 2 out of 8 unchallenged control monkeys also died of a "cholera-like illness."

[2] 2 out of 6 unchallenged control monkeys also died of a "cholera-like illness."

Table 2.—Isolations of *V. cholerae* from orally challenged monkeys

	Monkeys found dead		Sacrificed monkeys		Total	
	Number	Percent	Number	Percent	Number	Percent
Liver or bile	11/28	39	9/21	43	20/49	41
Stomach	18/20	90	7/17	41	25/37	68
Small intestine	57/62	92	36/51	70	93/113	83
Large intestine	48/56	85	20/41	49	68/97	70

TABLE 3.—The relationship of infected liver parenchyma or bile to time after challenge and to the presence of choleralike illness

	Time after challenge		Association with disease	
	8 days or less	More than 8 days	Cholera-like illness	No cholera
Liver:				
Positive	5	0	3	2
Negative	4	10	2	13
Bile:				
Positive	7	10	3	14
Negative	12	38	8	42

as were biopsies taken on September 21 and 24. Rectal swabs were positive from August 27 until September 12 and again on September 15, 17, and 18. Rectal swabs became positive again on September 22 and contained vibrios every day until November 15. Gastric aspiration on September 27 was positive for Inaba vibrios. Swabs taken after November 15 were predominantly negative. On November 19 the rectal swab was positive; on November 29 a stomach tube was passed and no vibrios were recovered, but rectal swabs were positive on that day and the following. On December 7 bacteriologically positive duodenal fluid was obtained; stools were positive on December 9, 11, 13, and 14 and from that time on they have been consistently negative, as were two gastric fluids obtained on January 7 and March 10, 1965.

(4) There was a significantly shorter duration of positive cultures in monkeys which had been previously challenged (table 4), indicating that previous challenges may confer some immunity.

TABLE 4.—Effect of prior exposure to *V. cholerae* on duration of positive rectal swab

	Duration positive rectal swabs		Total
	Less than 3 days	3 days or more	
First challenge	32	59	91
Subsequent challenge	32	14	46

DISCUSSION

Although it has not been possible to define the conditions under which a rhesus monkey will consistently develop cholera, some important observations have been made which may have correlates in human disease. First, the rhesus monkey can harbor large numbers of vibrios in the upper intestine and/or bile without having a positive rectal swab. This suggests a possible location for a silent reservoir in man. In monkeys such silent infection has continued for as long as 15 days after the last positive rectal swab. Second, both liver and bile infection can occur in spite of a severed bile duct, suggesting portal blood stream and/or a lymphatic infection. This could also be present in humans and may play a role in the pathogenesis of the disease.

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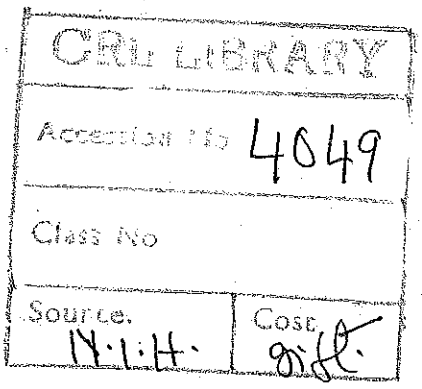

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