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Detection of *Vibrio cholerae* biotype *El Tor* by Purging *†

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Previous studies have demonstrated the value of purging in detecting inapparent cholera infection, but the technique has not been generally accepted. The present study shows that the method can be recommended as a routine procedure to determine when convalescent cholera patients should be discharged from hospital. It may also be useful in detecting carriers of Vibrio cholerae *and for evaluating chemotherapy. In this study, five of the eight patients who excreted vibrios after purging had been treated with 2 g of chloramphenicol daily for three days, a finding that throws doubt on the adequacy of this treatment. Purging with magnesium sulfate is simple, well tolerated and ideal for hospitalized patients or for subjects whose stools can be promptly cultured. Since magnesium sulfate is inhibitory to* Vibrio cholerae, *this purgative is not well suited for field studies, where there may be delays in culturing.*

The detection of inapparent infection in cholera was demonstrated by three separate studies of Tanda, Zirolia and Piras (cited by Pollitzer, 1959), who noted the reappearance of *Vibrio cholerae* in faecal specimens of convalescent cholera patients who were given magnesium sulfate and whose stool cultures had been negative on two or three previous examinations. In contrast, Gohar & Makkawi (1948), working in an Egyptian village, found magnesium sulfate of little value in detecting cholera carriers in " a few patients " whom they studied one month after an epidemic had ended. They could not recommend magnesium sulfate as a purgative in cholera, partly because of the *in vitro* inhibition of *Vibrio cholerae*. However, Lomberg (1950) found magnesium sulfate less inhibitory than sodium sulfate.

Hasan et al. (1965) postulated the possible existence of a reservoir in cholera on the basis of observations on laboratory-infected monkeys in which the upper small intestine was found to be culturally positive when the rectum and colon were repeatedly negative.

Purging is a useful and well-established technique for the detection of carriers of typhoid bacilli (Huckstep, 1962) and in amoebiasis (Castellani & Chalmers, 1919). We therefore decided to re-examine the question of purging to detect inapparent infection in cholera. Observations reported in the present study support the concept of a human reservoir in cholera and further indicate the value of purging to detect inapparent infection which escapes detection by routine stool or rectal swab examination. Thirty-eight convalescent cholera patients who had repeated negative daily stool cultures or rectal swabs were studied; eight (21 %) excreted vibrios after purging. These eight patients were investigated in detail.

METHOD

Three or four hours after breakfast, each patient was given a 30-g dose of magnesium sulfate in a glass of water. A watery stool was usually passed within one to four hours. Occasionally, no bowel movement followed the 30-g dose, in which case a 45-g dose was given the next day. With the patient's co-operation,

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an attempt was made to secure the last portion of the bowel movement, which presumably represented a sample from the upper gut. This specimen was inoculated directly on TCBS [1] medium (Kobayashi et al., 1963) by heavy streaking with a cotton swab. A tube of alkaline peptone water was also inoculated and was incubated for five hours. From this broth, a second TCBS plate was prepared. After 8-10 hours of incubation, *Vibrio cholerae* produced large, flat, moist, yellow colonies or a diffuse, yellow, mucoid growth with a characteristic sweet odour. Bacteriological diagnosis was confirmed by agglutination with specific typing sera and by biochemical tests.

Quantitative bacterial counts were obtained by using a tenfold dilution of stool in Feeley's gelatin-buffered saline (phosphate-buffered saline (pH 7.4) containing 0.1% gelatin) or alkaline peptone water. A small amount (0.1 ml) of stool suspension was spread on the surface of TCBS medium by means of a bent glass stirring-rod. In addition to undiluted stool, three dilutions were made—namely, 10^{-4}, 10^{-7}, and 10^{-9}. Colonies were counted after 18-24 hours of incubation at 37°C.

Stools were homogenized by repeated aspiration and discharge of suspended stool in test tubes; 1-ml pipettes were used. Because of the presence of mucus, particularly in " rice-water " stools, it was not always possible to obtain a completely homogeneous suspension. Usually, individual pipettes were used to prepare each tenfold dilution; occasionally, when pipettes were in short supply, as few as four pipettes were used for a complete serial dilution. For these reasons vibrio counts could only be approximated, particularly at the upper quantitative end-point.

All patients had daily stool cultures or, if a stool culture could not be obtained, a rectal swab was taken. Special care was taken to supervise the collection of stools and to inoculate specimens within one hour from the time of passage.[2] The infecting strain in seven of the eight cases was *Vibrio cholerae* biotype *El Tor*, Ogawa subtype; the Inaba subtype was responsible for infection in patient No. 6.

[1] Distributed by Tanabe Seiyaku Co. Ltd, 21 Doshomachi, 3-chome, Higashiku, Osaka, Japan. Names of commercial manufacturers and trade names are provided for identification only, and inclusion does not imply endorsement by the Public Health Service or the US Department of Health, Education, and Welfare.

[2] We have found that magnesium sulfate in large doses gives good results provided that the stool is cultured immediately after the purge. Magnesium sulfate is therefore not well suited for field studies in which there are long delays from the time specimens are obtained until they are cultured. Possibly other osmotic cathartics or purgatives may be preferable under field conditions.

CASE-HISTORIES

The eight patients who were found to excrete vibrios after purging had cholera gravis, manifested by vascular collapse, sunken eyes, moderate to marked dehydration, characteristic " washerwoman's " hands, cramps in the extremities, and aphonia. All had a brief history of diarrhoea followed by vomiting. None had been vaccinated. Patients 1 and 8 were opium addicts and had had gastrectomy for peptic ulcer four and eight years, respectively, before admission. All were promptly treated with intravenous fluid and electrolytes and responded well. Vibrios isolated (before and after treatment) from the first group of patients, who were given chloramphenicol for three days, were markedly sensitive to this drug when examined by the disk-sensitivity method.

Patients given chloramphenicol for three days with or without other chemotherapy.

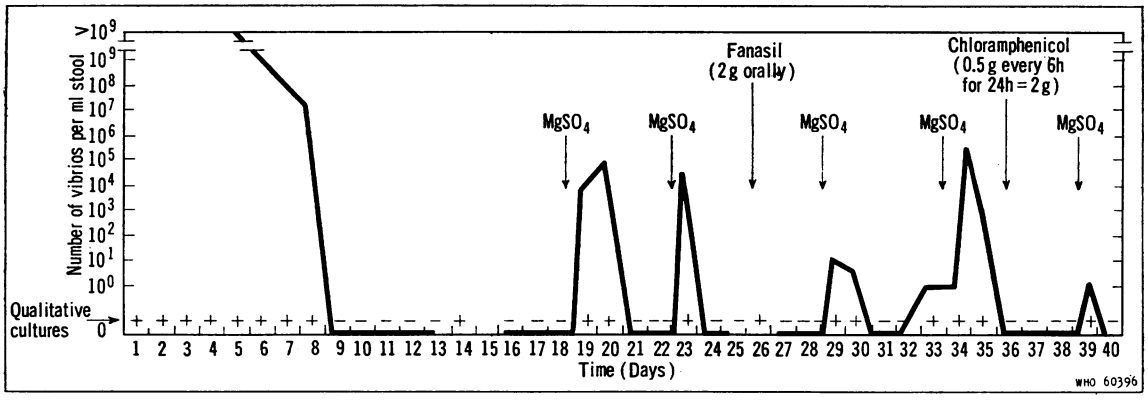
Patient No. 1. This patient was a 50-year-old male labourer. As Fig. 1 shows, he excreted large numbers of vibrios during the first eight days of hospitalization. On the ninth day, his stool culture was negative and remained so until the 14th day, the day designated arbitrarily for the discharge of patients who had at least three successive negative daily cultures. Stool culture on the 14th day was of considerable interest in that direct culture on TCBS medium revealed no vibrios; however, after a five-hour enrichment in alkaline peptone broth and subsequent growth on TCBS medium, two typical colonies appeared, which were confirmed as *Vibrio cholerae*, biotype *El Tor*, Ogawa subtype, the same strain that was present when the patient was admitted. It was this observation that raised the question of inapparent and persistent infection.

The patient was readmitted on the 16th day and was found to have no vibrios in his stool for three days. On the 19th day the stool culture was still negative, but purging with magnesium sulfate yielded large numbers of vibrios for two days. Thereafter the stool culture was negative until the second purge produced copious vibrios for one day. After two more days of negative cultures, a few organisms were passed spontaneously.

The patient was then given orally a single 2-g dose of 4-sulfanilamido-5,6-dimethoxypyrimidine [3] on the 26th day. He excreted no vibrios for the next two

[3] Fanasil (Roche).

FIG. 1
VIBRIO EXCRETION AFTER PURGING (PATIENT No. 1)



days. On the 29th day the stool culture was again negative, but purging for the third time revealed moderate numbers of vibrios for two days. Subsequently, the stool culture was negative until the patient spontaneously passed small numbers of organisms on the 33rd and 34th days, which were detected only after enrichment in alkaline peptone water.

On the 34th day, bile collected from the duodenum through a Crosby capsule biopsy instrument revealed 5×10^2 organisms; after oral ingestion of magnesium sulfate, the bile contained 5×10^3 vibrios, suggesting that the gall bladder may be a possible site of infection. The same bile specimens, collected before and after purging, were kept at room temperature for two hours and then stored in the refrigerator overnight; the following day a more detailed quantitative study was performed in which 0.1 ml from each tenfold dilution was cultured on TCBS. With regard to the bile specimen obtained after purging, there was less growth in the specimen stored in the presence of magnesium than in that cultured the previous day (7×10^1, compared to 5×10^3) and the colonies were small and atypical, indicative of the known inhibitory effect of magnesium (Gohar & Makkawi, 1948; Lomberg, 1950).

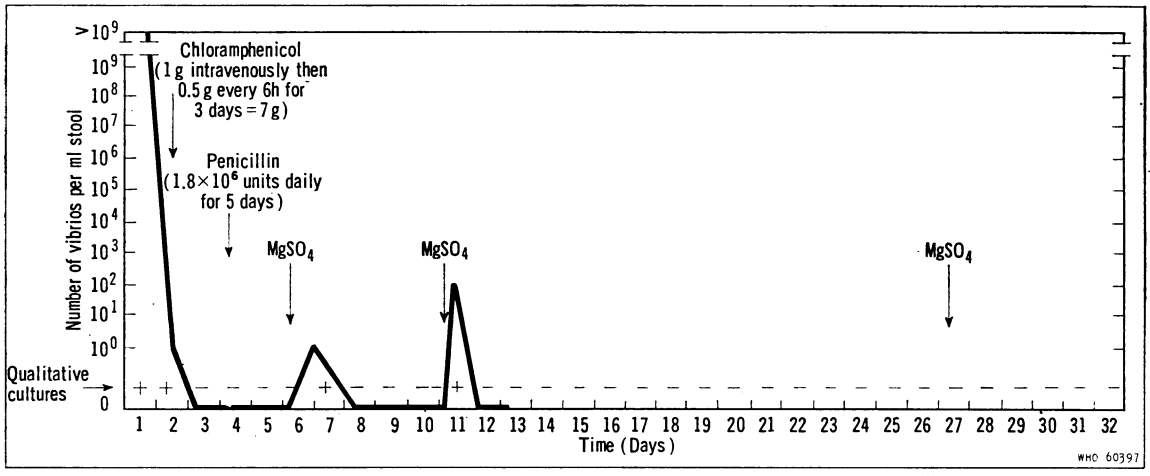
On the 36th day, the patient was given 0.5 g chloramphenicol every 6 hours for four doses, a total of 2 g. Two days later, purging for the fifth time again revealed small numbers of vibrios for one day. He then received 0.5 g chloramphenicol every 6 hours from days 40 to 42, a total of 6 g. Stool cultures were negative from the 41st to the 47th day. On the 48th day he was purged for the sixth time (not shown in Fig. 1) and again the stool was positive for two days. Observations continue.

Patient No. 2. The patient was a 16-year-old boxer. He was treated with chloramphenicol—1 g intravenously and then 0.5 g orally every 6 hours for three days, a total of 7 g. On this regime, his initial high stool-vibrio count promptly fell, and his stool culture was negative after 24 hours of treatment (Fig. 2). During treatment, and for 24 hours afterwards, his stool culture remained negative, until he was purged on the 6th hospital day, after which he excreted small numbers of vibrios for one day. He then had no vibrios in his stool for four days, until a second purge on the 11th hospital day resulted in the excretion of moderate numbers of vibrios for one day. There were no vibrios in his stool cultures for the next 14 days. He was purged for the third time on the 26th day, but no vibrios could be found. His stool culture remained negative through the 32nd hospital day.

Because of a wound infection in his cut-down site, he was treated with 1.8 million units of crystalline penicillin daily from the third to the seventh day. (Parenteral penicillin in three other patients did not eliminate vibrios or shorten the duration of excretion.)

Patient No. 3. This patient was a 35-year-old male labourer. He received 5 g of streptomycin orally in one dose, but the stool culture remained positive for the first three days of hospitalization. No quantitative studies were made. He was given 0.5 g chloramphenicol every 6 hours for 3 days (total, 6 g), beginning on the third hospital day. On the fourth day the stool was still positive, but no vibrios could be found on the fifth to the seventh days. On the

FIG. 2
VIBRIO EXCRETION AFTER PURGING (PATIENT No. 2)



eighth day, three days after chloramphenicol treatment ended, the stool culture was negative; he was purged, which resulted in the excretion of vibrios for one day. His stool cultures were negative from the ninth through the 17th day.

Patient No. 4. This patient was a 32-year-old housewife. She received 0.5 g chloramphenicol every 6 hours for her first three days in hospital. The stool culture promptly became negative and remained so from the second to the 10th day. On the 10th day, she was purged and was found to excrete vibrios for one day. Stool cultures were then negative from the 11th through the 14th day. The second purge on the 15th day was negative and the stool culture remained negative on the 16th day, after which she was discharged.

Patient No. 5. This patient was a 60-year-old farmer. He received 0.5 g chloramphenicol every 6 hours for the first four days of hospitalization (total, 8 g). Through an error, no culture was obtained until the fourth day, when the stool was found to be positive, although the patient was still receiving chloramphenicol. Stool cultures were negative on the fifth to the eighth days. On the eighth day, he was purged and was found to excrete vibrios for one day. Observations continue.

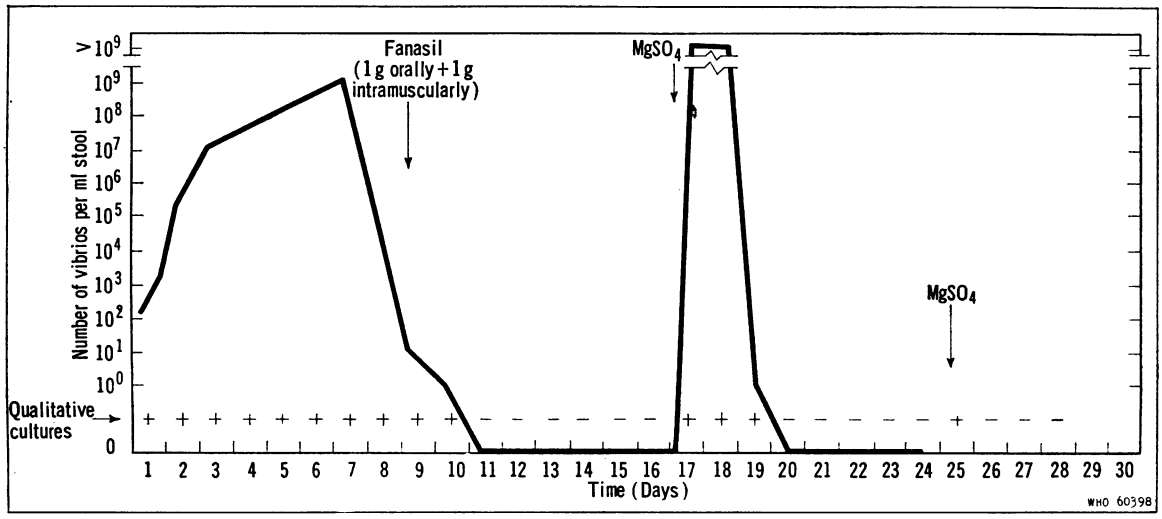
Patients who did not receive chloramphenicol

Patient No. 6. The patient was a 55-year-old seamstress. During the first eight days of hospitalization, she was treated with fluids and electrolytes only; on the ninth day, the diarrhoea was much worse and she suddenly went into shock. For this reason, she was rehydrated and treated with Fanasil, 1 g orally and 1 g intramuscularly being given simultaneously. She excreted vibrios during the first 10 days of hospitalization (Fig. 3) but the stool culture was negative for the next six days. Purging on the 17th day revealed a larger number of vibrios for two days than at any time previously, and the stool remained positive for three days. Stool cultures were negative from the 20th through the 24th day. She was purged for the second time on the 25th day and again the stool culture was positive for one day (no quantitative study was made). The stool culture was then negative from the 26th through the 28th day. Observations continue.

Patient No. 7. The patient was a 25-year-old male labourer. He responded well to conventional fluid and electrolyte therapy without chemotherapy. He excreted large numbers of vibrios for the first few days of hospitalization, then gradually fewer until the beginning of the eighth day, when his stool culture was negative and remained vibrio-free for nine days. On the 16th hospital day, he was purged; moderate numbers of vibrios were excreted in the stool. Unfortunately, he left hospital and could not be studied further.

Patient No. 8. The patient was a 45-year-old male peddler. He responded well to conventional fluid and electrolyte therapy without chemotherapy. He excreted large numbers of vibrios during the first five

FIG. 3
VIBRIO EXCRETION AFTER PURGING (PATIENT No. 6)



days of hospitalization. The stool culture then became negative for four successive days. On the 10th hospital day, the patient was purged, after which he excreted moderate numbers of vibrios in his stool. Unfortunately, he left hospital and could not be studied further.

DISCUSSION

The suggestion, based on the results of this study, that the gall bladder is a possible site of infection, is in line with the autopsy observations of numerous workers (cited by Pollitzer, 1959), in which the gall bladder and biliary system were frequently found to harbour vibrios. More recently, Hasan et al. (1965) observed that the upper small intestine was culturally positive when the rectum and colon were repeatedly negative for vibrios in laboratory-infected monkeys.

It is possible that, in the carrier state, vibrios are shed intermittently and in small numbers from the upper bowel. Under normal circumstances, vibrios may not survive in the large bowel in the presence of large numbers of more successful competitors, such as *Escherichia coli* (Freter, 1956; Ransom et al., 1961). It is useful to think of the normal flora as a biological filter, which eliminates these pathogenic vibrios if there is sufficient contact, as in the normal lower bowel. This may explain why rectal swabs and stool cultures are frequently negative in these convalescent patients and, presumably, in other carriers. However, when the organisms are flushed from their focus in the upper gut or gall bladder by a cathartic, or perhaps in intermittent diarrhoea from other causes, organisms may appear in the stool in large numbers. This may well occur commonly and may be the mechanism for the persistence of cholera in an endemic focus. The quantitative examination of vibrios in stools suggests that apparently healthy intermittent carriers may be important in the transmission of cholera, particularly when there is diarrhoea from any cause. The need to find, study, and treat such carriers is clear. Cholagogue purging seems to be promising as a means to this end.

Several important questions arise from this study. Does vaccination protect against persistent or chronic infection? None of the eight patients discussed in this report had been vaccinated; however, of the 30 others in this study (who were not found to harbour vibrios after purging), only three had been vaccinated twice—the other 27 had had no vaccination. Therefore, the present data do not provide an answer to this important question.

Exactly what mechanisms are involved in recovery from cholera? If an immune mechanism, such as coproantibody production, is important, what defect explains the persistence of infection demonstrated by purging? Why do antibiotic drugs, which are so effective *in vitro*, fail to eliminate vibrios *in vivo* in those patients with persistent infection? Are there

pre-existing abnormal pathophysiological conditions, such as gastritis, acute hypoacidity, or abnormal motility (suggested by the opium addiction encountered in this study), which predispose to cholera? Two of the eight subjects in this small series had had a gastrectomy. Two other individuals with gastrectomy were observed in a total of 120 bacteriologically proved cases. This is surprising, considering the limited access that people of this socio-economic group have to medical care. Is there a difference in the intestinal localization of vibrios in the acute form of the disease compared with the convalescent carrier state?

The finding of vibrios after purging in five patients who had received a full three-day course of chloramphenicol treatment raises the question of the adequacy of this treatment regime—perhaps a longer treatment or other drugs may be required. It would seem desirable to determine the optimum antibiotic treatment for cholera by comparing inapparent infection rates determined by purging in convalescence.

At most treatment centres, it is customary to discharge patients after three negative stool cultures or rectal swabs on successive days during convalescence. It can be inferred from this study that this is inadequate and that purging may be a more useful method to assess the public health liability of convalescent cholera patients. The technique, which is simple and well tolerated, has already been adopted in one treatment centre in Iran as a routine procedure to determine when cholera patients should be discharged.

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RÉSUMÉ

Bien que plusieurs expérimentateurs n'aient pu réussir à dépister les infections cholériques inapparentes par culture des selles obtenues après purgation au sulfate de magnésium, une nouvelle tentative a été faite chez 38 convalescents de choléra à *Vibrio cholerae* biotype *El Tor*.

Une selle liquide a été généralement obtenue une à deux heures après administration de 30 g de sulfate de magnésium dans un verre d'eau ou de 45 g le jour suivant lorsque la première prise n'avait pas agi. On s'est efforcé de recueillir la dernière partie de l'évacuation provenant vraisemblablement de la portion haute de l'intestin. Cet échantillon a été ensemencé directement sur milieu TCBS et sur un tube d'eau peptonée alcaline incubée pendant 5 heures. A partir de celle-ci une seconde boîte de milieu TCBS a été préparée. En 8-10 heures, *V. cholerae* donne des colonies caractéristiques; le diagnostic bactériologique est confirmé par l'agglutination avec des sérums spécifiques et l'étude des réactions biochimiques.

Les huit malades qui ont éliminé des vibrions après purgation avaient été atteints d'une forme grave de choléra; tous avaient eu un épisode diarrhéique suivi de vomissement. Aucun n'était vacciné. Ils avaient bien réagi au traitement réhydratant et cinq d'entre eux avaient reçu 2 g de chloramphénicol par jour pendant trois jours.

Les résultats de cette expérimentation suggèrent que la vésicule biliaire et la portion haute de l'intestin peuvent représenter un foyer d'infection chronique qui ne peut être décelé par les examens de selles courants. La règle qui consiste à faire sortir un malade de l'hôpital après trois coprocultures négatives, à des jours successifs, paraît insuffisante aux auteurs qui conseillent de compléter cet examen par une coproculture après purgation.

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