Discussion. Gross steatorrhea in otherwise healthy subjects was induced by administration of bile acid sequestrant in appropriate dosage. Such steatorrhea was associated with deficient digestion and absorption of ingested triglyceride as shown by fat balance studies and I^{131}-triolein studies. In contrast, there was no interference with absorption of I^{131}-oleic acid during period of resin administration. These studies suggest that the mechanism of resin-induced steatorrhea is related to exclusion of bile acids from participation in hydrolytic digestion of dietary triglyceride.

Malabsorption has been reported following administration of neomycin(4), and trimethyl hexadecyl ammonium stearate(5). In the former type large doses of neomycin were required and steatorrhea was inconsistent. In the latter, chemical used was very toxic to animals, producing moderate to severe damage of intestinal villi. In contrast, use of MK-135 appears to be an innocuous and effective method for induction of steatorrhea in man.

Summary. Gross steatorrhea has been induced experimentally in healthy human subjects by administration of a resin capable of sequestering bile acids in the intestinal lumen. This agent (MK-135) inhibited absorption of I^{131}-labeled triolein but not I^{131}-labeled oleic acid. In contrast to certain other forms of experimental malabsorption, MK-135-induced steatorrhea appears to be predictable and innocuous.

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Antiphage Activity of Human Cholera Sera. (26276)

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Although Doorenbos and Cossery(1) have reported on the inactivation of cholera phage by rabbit antisera, a search in pertinent literature failed to find evidence of studies concerned with the anticholeraphage activity of human sera. It was believed, therefore, that such an investigation of the sera from cholera patients might yield significant results.

Materials and procedures. One hundred seventy-seven sera collected during the 1958-1960 cholera epidemic in Thailand and preserved in the frozen state at −20°C were used. There were 37 pairs of sera from cholera patients. One sample was drawn during the first or second day of the disease, while the other was collected 12 to 24 days later. Eighteen paired sera were obtained from symptomless carriers of *V. comma*; 20 from contacts who had no clinical signs of cholera and from whose stools *V. comma* was not isolated; and 30 from patients with acute shigellosis or salmonellosis. All were collected in 12 to 25 day intervals. Thirty-five unpaired sera were from apparently healthy soldiers of the Royal Thai Army. Approximately 90% of all sera, including those from bacteriologically proven cases of cholera, came from individuals who were recently vaccinated against cholera.

A bacteriophage, isolated from a canal in Bangkok, which belonged to the first group according to the classification of Mukerjee

et al.(2) was employed, together with a *V. comma Ogawa* strain cultured from a classic case of cholera in Bangkok which was classified by Dr. Mukerjee as phage type 1.

The propagation of the phage and of the Ogawa organism was carried out according to Mukerjee *et al.*(2), while evaluation of the inhibitory power of the sera was undertaken by closely following the method of Toussaint and Muschel(3). The phage was used in a dilution of 10^{-3}. Two-fold serial dilutions of the sera, from 1:10 to 1:320, were employed. The phage-serum mixtures were incubated for 2 hours at 37°C before addition to the vibrio strain. The highest dilution of sera causing at least 50% inactivation was considered the titer. Paired sera from the same individual were tested on the same day.

Results. All tested sera at 1:10 dilution produced at least a 50% inhibition of the activity of the diluted cholera phage. Those collected during the acute cholera, from carriers, contacts, noncholeraic diarrhea and from symptomless soldiers never showed such activity in dilutions greater than 1:80. The second sera from carriers, contacts and noncholeraic diarrhea either inactivated cholera phage in the same dilution as the first sera (49 out of 68) or in the next higher (9 out of 68) or the next lower (6 out of 68) dilution. Only 4 of the second sera in this group showed a greater increase or decrease in the titer. On the other hand, sera drawn 12 to 24 days after onset of clinical cholera demonstrated an antiphage activity in at least 1:20 and in dilutions as high as 1:320. Average increase in the titer of the convalescent over the acute specimens was approximately 3-fold. The results in patients with cholera are demonstrated in Table I.

Discussion. Inhibition of phage activity by normal sera had been demonstrated by Toussaint and Muschel(3) who used bacteriophage of the *E. coli* T series. The work reported here indicates that a cholera phage of type I is also susceptible to inactivation by sera. It is possible that human sera in low dilutions will inactivate many of the phages which lyse enteric bacteria.

A significantly greater antiphage activity was observed after convalescence from cholera and may be related to increased levels of phage antibody.

It is well known that cholera phage is excreted at the end rather than at the beginning of cholera(4). Thus the increase in antiphage activity after illness is not surprising.

Keller and Engley(5) and Keller and Zatzman(6) showed that phage disappears from the bodies of experimental animals quite rapidly. It is not known, however, if cholera phages ever enter the human circulation. Thus one can only conjecture whether the classical antibody-complement system or other serum components are involved in anticholera-phage activity. One has to note, however, that an increase in antiphage activity occurred only in the sera of those individuals with clinical disease and not during the carrier status nor in exposed persons who undoubtedly ingested *V. comma* at one time or another. The increased antiphage activity is thus closely associated with clinically manifest cholera. Obviously further studies of phage neutralizing activity of body fluids in health and sickness are required in cholera and in other diseases.

TABLE I. Anticholeraphage Titers of Sera from Cholera Patients.

No. of patients	Reciprocal titer during acute illness	Reciprocal titer after convalescence in No. of patients				
		20	40	80	160	320
23	20	2	4	12	2	3
12	40	1	1	2	5	3
4	80	0	1	1	0	2

Summary. Increased anticholeraphage activity of human sera was observed after cholera but not in carriers, contacts and healthy controls.

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Cholesterol-4-C^{14} and Bile Acids in the Guinea Pig.* (26277)

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Cholic acid (3*a*, 7*a*, 12*a*-trihydroxycholanic acid) has been isolated from the bile of many animal species. Because of the reported absence of 3*a*, 7*a*, 12*a*-trihydroxycholanic acid in the bile, the guinea pig has been termed "unique"(1). Recently we have found that 3*a*, 7*a*, 12*a*-trihydroxycholanic acid is the most abundant bile acid in the adult guinea pig, although absent in the immature animal(2). In determining the bile acid pattern of a particular species, several aspects have emerged: a) conversion of sterols into several bile acids having species-variable interconversion(1), b) oxidation of side chain in relation to 12-hydroxylation, and c) modification of hydroxyl groups during enterohepatic cycling(3). In studies on formation of bile acids the bile has been obtained from gall bladders and from biliary tract fistulae. In preliminary studies we found that the voluminous flow of bile from biliary fistulae resulted in early death of the guinea pigs. Recycling of bile or replacement of bile removed from the catheterized biliary tract permitted long term survival of the guinea pigs and the present study of conversion of cholesterol-4-C^{14} into labeled bile acids under favorable physiological conditions and at different phases of the enterohepatic cycling.

Methods. Young adult guinea pigs of either sex, approximately 5 months of age and weighing 500-700 g, were used in this study. A bile fistula was performed as described previously(2) in all animals. Forty-eight hours after operation, cholesterol-4-C^{14} was injected into the ear vein. For study of continuous enterohepatic cycling the catheters remained connected and small samples of bile were removed periodically. For study of freely flowing bile the 2 catheters were not connected until the ninth hour after administration of cholesterol-4-C^{14}. Aliquots of the samples of the free-flowing bile collected from 0-1, 1-3, and 3-6 hours after injection were given to different guinea pigs intraduodenally *via* common bile duct catheter (Fig. 1). The free-flowing bile from these animals was then collected for 9 hours. In all studies of free biliary flow a volume of normal unlabeled bile equal to that diverted was supplied through the duodenal fistulae. After 9 hours of free flow the catheters were connected and thereafter small daily samples of bile were taken in all animals.

For the continuous enterohepatic cycling 0.450 μC (29.5 μg), and for 9 hours free flow 0.900 μC (59 μg) were used. Radioactivity in the bile samples infused intraduodenally into other guinea pigs was: 0-1 hr, 0.0651 μC; 1-3 hr, 0.0671 μC; 3-6 hr, 0.0334 μC.

Bile acids were separated and identified by procedures used previously(2). After removal of the conjugated bile acids from the columns, residual radioactive materials ("neutral sterols") were eluted with chloroform. Aliquots of the initial bile samples, extracts and fractions of column effluents were plated on aluminum planchets and C^{14} determined with a windowless flow counter with sufficient accumulated counts per sample for 1% standard error. The paper chromatograms were scanned for radioactivity

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