

EXPERIMENTAL CHOLERA-CARRIERS *

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The following series of experiments was undertaken to determine definitely whether animals could be made carriers of the cholera vibrios, the length of time they would remain carriers, and the nature of the condition, the object being to subject such experimental carriers to various methods of treatment, some of which have been recommended in practice, while others are based on theory.

TECHNIC

Inoculation.—The culture used, isolated during the recent outbreak of cholera in Austria, was furnished by Karl Landsteiner of Vienna, and was known at the laboratory as Cholera Austria 4. The culture was inoculated into ox bile and transplanted daily for a week before the experiments were begun, as cholera vibrios had been observed to remain alive in bile for a considerable length of time, in lively motility.

In the inoculation of the gall-bladder, the stomach, and the intestine, rigid antiseptic measures were adopted to avoid accidental infection. The animals were shaved, the skin washed with lysol solution, and then painted with tincture of iodine. For an inoculation of the stomach or of the small intestine, the abdominal cavity was opened in the middle line in the epigastric region.

The gall-bladder in guinea-pigs is rather free and of comparatively large size. It is located between the lobes of the liver (only a small part of it being attached to the liver), in the angle formed by the right costal margin and the xiphoid process. In performing the operation an incision was made from the middle of the xiphoid process to the right costal margin. As soon as the muscles were separated, the peritoneum became visible, and through it the xiphoid process. The latter was clasped with a hemostat and lifted up, whereupon the duplicature of the peritoneum formed thereby was perforated by means of a dull forceps. As a rule, the gall bladder was immediately visible and, prolapsing into the laparotomy-wound, it closed the opening, thus preventing exposure of the other organs. The injection could be made therefore outside the peritoneal cavity. Only fractions of a cubic centimeter were injected into the gall-bladder. The inoculation finished, the puncture in the gall-bladder was closed with a ligature, the ends of the wound were lifted up, and the gall-bladder assumed its normal position. Silk threads thoroughly soaked with tincture of iodine, were used for suturing the abdominal wound.

Intravenous inoculation was made in the usual way.

For inoculation by feeding the animal was held on a tray in frog position with head up. The outlet of a graduated pipet containing the bacterial suspension was placed in its mouth and the suspension allowed to flow into the pharynx. Tame animals, if handled cautiously, will take several cubic centi-

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meters of liquid without spilling a drop. The feeding finished, the mouth of the animal was wiped off with a piece of cotton soaked in lysol and the tray thoroughly disinfected.

Inoculated animals were kept either in glass jars or in solid galvanized-iron cages. These were disinfected at regular intervals. The feeding of the animals, and the cleaning and disinfection of the containers, were attended to by the author.

Examination.—To ascertain whether or not the animals became infested with cholera vibrios they were killed and examined immediately. The shaved skin over the chest and abdomen was wetted with 2% lysol solution. The abdomen and thorax were opened separately. The gall-bladder including the bile duct, the stomach, the proximal part of the intestine (in the reports, duodenum), the distal part of the small intestine (in the reports, the ileum), the cecum, and the distal part of the large intestine (in the reports, rectum) were double ligated, and then removed from the abdomen—first the gall-bladder, then the duodenum, ileum, stomach, cecum, and rectum, in order, a separate set of sterilized instruments being used for each organ. The gall-bladder was taken out in toto, including a portion of the bile duct. The contents of the gall-bladder were emptied into peptone water, and the gall-bladder together with the bile duct placed in a culture tube. The contents of the proximal part of the small gut were planted, and the intestine, cut into small pieces, was added to the same peptone tube. At least one-half of the distal part of the small intestine was planted in the same way as the duodenum. The stomach was opened on the large curvature, and 5 large loopfuls of the contents inoculated into peptone water. The same amount of the contents of the cecum was planted in a similar way. One portion of formed feces obtained from the distal part of the large intestine, was crushed with sterile forceps and placed in peptone water. During life, shortly after feeding, the animals were placed in thoroughly disinfected glass jars, and the feces collected as soon as deposited, peptone cultures being made in the same way as that described.

The peptone cultures thus obtained were sub-planted, after from 6 to 8 hours' incubation, into another set of tubes containing peptone water. The first and second peptone cultures were examined microscopically from time to time and plated on Dieudonné medium after 12 and 18 hours, respectively.

INTRAVESICULAR INOCULATION

Four guinea-pigs were inoculated in the gall-bladder. An alkaline-agar subculture was used, 0.1 of one slant being injected.

GUINEA-PIG 1.—Killed and examined 24 hours after inoculation. Peptone cultures from gall-bladder, ileum, and cecum were positive for cholera vibrios.

GUINEA-PIG 2.—Killed and examined 2 days after inoculation. Peptone cultures from gall-bladder, duodenum, and ileum were positive; the culture from the cecum was negative.

GUINEA-PIG 3.—Killed and examined 4 days after inoculation. Peptone cultures from gall-bladder, duodenum, ileum, and cecum were positive.

GUINEA-PIG 4.—Killed and examined 7 days after inoculation. Peptone cultures from the gall-bladder, duodenum, ileum, and cecum were positive; those from stomach and rectum were negative.

Four guinea-pigs inoculated in the gall-bladder harbored cholera vibrios. The animals were examined 1, 2, 4, and 7 days after inoculation.

INOCULATION OF THE STOMACH

Five guinea-pigs were inoculated in the stomach. Magnesium oxid suspended in water was given by mouth previous to the operation. Cholera Austria 4 was used, 0.025 of a slant being injected. The culture had been transplanted daily in ox bile for a week, passed through one rabbit, and recovered from the gall-bladder of the animal 13 days after inoculation.

GUINEA-PIG 5.—Examined 2 days after inoculation. It had died of pneumonia. Peptone cultures from gall-bladder, stomach, duodenum, ileum, and rectum were positive.

GUINEA-PIG 6.—Examined 3 days after inoculation. Peptone cultures from gall-bladder, duodenum, ileum, and cecum were positive.

GUINEA-PIG 7.—Examined 4 days after inoculation. It had died of pneumonia. Peptone cultures from gall-bladder, stomach, duodenum, and ileum were positive; those from cecum and rectum were negative.

GUINEA-PIG 8.—Examined 7 days after inoculation. Peptone cultures from gall-bladder, stomach, duodenum, ileum, and cecum were negative.

GUINEA-PIG 9.—Examined 8 days after inoculation. Peptone cultures from gall-bladder, duodenum, and ileum were positive; those from stomach, cecum, and rectum were negative.

Four of the 5 guinea-pigs inoculated, harbored cholera vibrios 2, 3, 4, and 8 days after inoculation. One animal of 5 was negative 7 days after inoculation.

INOCULATIONS OF THE SMALL INTESTINE

Five guinea-pigs were inoculated in the small intestine after the same method as in the case of the stomach.

GUINEA-PIG 10.—Examined 2 days after inoculation. Peptone cultures from gall-bladder, duodenum, ileum, and cecum were positive.

GUINEA-PIG 11.—Examined 7 days after inoculation. Peptone cultures from gall-bladder, duodenum, ileum, and cecum were negative.

GUINEA-PIGS 12 AND 13.—Examined 8 days after inoculation. Peptone cultures from gall-bladder, stomach, duodenum, ileum, and cecum were negative.

GUINEA-PIG 14.—Examined 10 days after inoculation. Peptone cultures from gall-bladder, duodenum, ileum, stomach, and cecum were negative.

One of the 5 animals inoculated in this manner harbored cholera vibrios 2 days after inoculation. Four of the 5 guinea-pigs, examined 7, 8, and 10 days after inoculation, were negative.

INOCULATION BY FEEDING

Five guinea-pigs were inoculated by feeding. The culture, Cholera Austria 4, had been transplanted daily in ox bile for a week and passed through one guinea-pig. One tenth of a slant was fed, magnesium oxid being given with the culture.

GUINEA-PIGS 15, 16, AND 17.—Examined 3 days after inoculation. Peptone cultures from gall-bladder, stomach, duodenum, ileum, and cecum were negative.

GUINEA-PIGS 18 AND 19.—Examined 4 days after inoculation. Peptone cultures from gall-bladder, stomach, duodenum, ileum, and cecum were negative.

Not one of the 5 animals, examined 3 and 4 days after inoculation, harbored cholera vibrios.

Three guinea-pigs, unfed for 18 hours, were given magnesium oxid by mouth. One and a half hours later they were fed 2 c.c. of culture, Cholera Austria 4, grown in ox bile for 24 hours. (The culture had been transplanted daily in ox bile and passed through one guinea-pig.) All the animals became sick, took little food, and had diarrhea.

GUINEA-PIG 20.—Examined 20 hours after inoculation. Intestine distended; contained bile. Gall-bladder about twice the normal size. Peptone cultures from gall-bladder were negative; those from stomach, duodenum, ileum, and cecum were positive.

GUINEA-PIG 21.—Examined 3 days after inoculation. The gall-bladder was distended to twice the normal size. Peptone cultures from duodenum, ileum, and cecum were positive; those from gall-bladder and rectum were negative.

GUINEA-PIG 22.—Examined 7 days after inoculation. The gall-bladder was of normal size. Peptone cultures from gall-bladder, stomach, duodenum, ileum, and cecum were negative.

This experiment was repeated, each guinea-pig receiving one slant of agar culture suspended in alkaline peptone solution. The culture had been transplanted daily in ox bile, passed through one guinea-pig, and recovered from the gall-bladder of the animal 4 days after inoculation.

GUINEA-PIG 23.—Examined 20 hours after inoculation. Peptone cultures from ileum and cecum were positive; those from gall-bladder, stomach, and duodenum were negative.

GUINEA-PIG 24.—Examined 3 days after inoculation. Peptone cultures from gall-bladder, stomach, duodenum, ileum, and cecum were negative.

GUINEA-PIG 25.—Examined 7 days after inoculation. Peptone cultures from gall-bladder, duodenum, and ileum were positive; those from stomach, cecum, and feces were negative.

The experiment was again repeated. The culture had been transplanted daily in ox bile for a week, passed through 2 guinea-pigs, and recovered from the gall-bladders of the animals 4 and 7 days after

inoculation. In the experiment each animal received one agar slant suspended in alkaline peptone water.

GUINEA-PIG 26.—Examined 3 days after inoculation. The peptone culture from the ileum was positive; cultures from stomach, gall-bladder, duodenum, cecum, and feces were negative.

GUINEA-PIG 27.—Examined 8 days after inoculation. Peptone cultures from gall-bladder, stomach, duodenum, ileum, cecum, and feces were negative.

GUINEA-PIG 28.—Examined 14 days after inoculation. Peptone cultures from gall-bladder and ileum were positive; those from stomach, duodenum, cecum, and feces were negative.

From the last 3 experiments it is seen that the gall-bladder in animals which were killed and examined early after inoculation by feeding was free from cholera vibrios. Those animals which were killed and examined at later periods after inoculation, if they harbored cholera vibrios at all, showed them present in the gall-bladder.

INOCULATION BY INTRAVENOUS INJECTION

Two guinea-pigs were inoculated by intravenous injection. The culture, Cholera Austria 4, had been transplanted daily in ox bile, passed through 2 guinea-pigs, and recovered from the gall-bladders of the animals 4 and 17 days after inoculation. In the experiment a minute amount of cholera culture was injected. The animals were sick after the injection, but recuperated in 24 hours.

GUINEA-PIG 29.—Examined 4 days after injection. Peptone cultures from gall-bladder, stomach, duodenum, ileum, cecum, and feces were negative.

GUINEA-PIG 30.—Examined 12 days after inoculation. Peptone cultures from stomach, gall-bladder, duodenum, ileum, cecum, and feces were negative.

Four guinea-pigs (64, 65, 66 and 67) were inoculated by intravenous injection of 0.1 of a slant, Cholera Austria 4 being used. (The culture had been transplanted daily in ox bile for a week, passed through 6 guinea-pigs, and recovered from the gall-bladders of the animals, 7, 17, 6, 13, 1, and 5 days after inoculation.) The animals became sick after the injection and were found dead the next morning. Peptone cultures made from heart blood, spleen, lung, liver, gall-bladder, ileum, and cecum were positive for cholera vibrios.

An attempt to infest guinea-pigs by intravenous injection of small and large amounts of living cholera vibrios failed.

INOCULATION BY INJECTION OF CULTURE INTO THE SEROUS CAVITY

Three guinea-pigs were inoculated by injection of the culture into the right pleural cavity, Cholera Austria 4, 0.1 of one slant, being used.

The culture had been transplanted in ox bile daily for a week, passed through 6 guinea-pigs, and recovered from the gall-bladders of the animals 7, 17, 6, 13, 1, and 5 days after inoculation.

GUINEA-PIG 71.—Killed in ultimis and examined 24 hours after inoculation. Peptone cultures from pleura, peritoneum, blood, lungs, spleen, and liver were positive.

GUINEA-PIG 72.—Examined 2 days after inoculation. Peptone cultures from pleura, peritoneum, blood, lungs, spleen, liver, gall-bladder, duodenum, ileum, and cecum were negative.

GUINEA-PIG 73.—Examined 5 days after inoculation. Peptone cultures from pleura, peritoneum, blood, lungs, spleen, liver, gall-bladder, duodenum, ileum, and cecum were negative.

Of all the modes of inoculation the intravesicular injection showed the highest percentage of "takes." This method was adopted in the further course of the investigation.

TABLE 1
THE DISTRIBUTION OF CHOLERA VIBRIOS IN THE ALIMENTARY SYSTEM OF GUINEA-PIGS AND
THE DURATION OF THE CARRIER STATE AFTER INTRAVESICULAR INOCULATION

Guinea-pig	Days After Inoculation	Gall-bladder	Stomach	Duodenum	Ileum	Cecum	Rectum
1	1	+	0	0	+	+	0
2	2	+	0	0	+	+	0
62	3	+	—	+	+	+	0
68	3	+	—	+	+	+	0
69	3	+	+	+	+	+	+
3	4	+	0	+	+	+	0
56	4	+	—	+	+	+	+
65	4	+	0	+	+	+	—
49	5	+	—	+	+	+	—
50	5	+	—	+	+	+	—
63	5	+	—	+	+	+	—
34	6	+	—	+	+	—	—
40	6	+	0	+	+	—	—
41	6	+	0	+	+	+	—
54	6	+	—	+	+	—	—
55	6	+	—	+	+	+	—
57	6	+	—	+	+	+	—
58	6	+	—	+	+	—	—
4	7	+	—	+	+	+	—
35	7	+	—	+	+	—	—
59	7	+	—	+	+	—	—
66	8	+	—	+	+	+	—
67	8	+	—	+	+	+	—
70	8	+	—	+	+	+	—
32	10	+	—	+	+	+	—
60	10	+	—	+	+	+	—
33	11	+	—	+	+	+	—
46	13	+	—	—	+	+	—
47	13	+	—	+	+	+	—
48	13	+	—	+	+	+	—
64	16	—	—	—	—	—	—
36	17	+	—	+	+	+	—
43	30	—	—	—	—	—	—

+ = cholera vibrios present in the culture.

— = cholera vibrios absent in the culture.

0 = not examined.

The results of the examinations made on 33 experimental cholera-carriers are summarized in Table 1, which shows also the percentage

of "takes," the distribution of cholera vibrios throughout the various parts of the alimentary canal, and the duration of the condition in question.

The excretion of cholera vibrios in the feces of the animals deserves special mention. It will be seen from the examples of examination of feces for the presence of cholera vibrios that the successfully infested animals do excrete cholera vibrios in their feces. It is probably due to the peculiar structure of the gut and to the slight resistance of the cholera vibrio to unfavorable conditions that it does not occur as regularly as one would expect. Guinea-pigs 74, 76, and 77 serve as examples of the fact, already known with regard to human beings, that cholera vibrios can pass through the entire alimentary canal without getting a foothold in the body.

EXAMPLES OF EXAMINATION OF FECES FOR THE PRESENCE OF CHOLERA VIBRIOS
DURING THE LIFE OF EXPERIMENTAL GUINEA-PIG CHOLERA-CARRIERS

43.—Intravesicular inoculation. On the 8th, 9th, 10th, 11th, and 12th days after inoculation, negative; on 13th day, positive; on 15th, 16th, 17th, 18th, and 20th days, negative.

43a.—Intravesicular inoculation. On the 1st day, negative; on the 2nd, 3rd, and 4th days, positive.

44.—Intravesicular inoculation. On the 1st day, negative; on the 2nd, 3rd, and 4th days, positive.

45.—Intravesicular inoculation. On the 1st, 2nd, 3rd, 4th, 6th, 9th, 10th, 12th, and 13th days, negative.

46.—Intravesicular inoculation. On the 1st, 2nd, 5th, 8th, and 9th days, negative; on the 10th day, positive; on the 12th day, negative.

47.—Intravesicular inoculation. On the 1st, 2nd, 5th, 7th, 8th, 9th, and 10th days, negative; on the 12th day, positive.

48.—Intravesicular inoculation. On the 1st, 2nd, 5th, 6th, 8th, and 10th days, negative; on 12th day, positive.

49.—Intravesicular inoculation. On the 1st, 2nd, and 5th days, negative.

50.—Intravesicular inoculation. On the 1st day, negative; on the second day, positive; on the 5th day, negative.

60.—Intravesicular inoculation. On the 3rd day, positive; on the 4th day, negative.

63.—Intravesicular inoculation. On the 4th day, negative.

64.—Intravesicular inoculation. On the 2nd, 4th, 6th, 8th, and 9th days, negative.

65.—Intravesicular inoculation. On the 2nd and 3rd days, negative.

66.—Intravesicular inoculation. On the 3rd day, positive.

67.—Intravesicular inoculation. On the 2nd day, positive.

68.—Intravesicular inoculation. On the 2nd day, negative.

69.—Intravesicular inoculation. On the 2nd day, positive.

70.—Intravesicular inoculation. On the 2nd day, negative.

74.—Inoculation by feeding. On the 1st day, positive; on the 2nd and 3rd days, negative. Animal killed. Gall-bladder, stomach, and intestines negative.

75.—Inoculation by feeding. On the 1st, 2nd, and 3rd days, negative. Animal killed. Gall-bladder, stomach, and intestines, negative.

76.—Inoculation by feeding. On the 1st day, positive; on the 2nd and 3rd days, negative. Animal killed. Gall-bladder, stomach, and intestines, negative.

77.—Inoculation by feeding. On the 1st day, positive; on the 2nd and 3rd days, negative. Animal killed. Gall-bladder, stomach, and intestines negative.

SUMMARY

Attempts were made to produce in animals a condition which would resemble that of cholera-carriers in human beings. Inoculations of cholera vibrios into the gall-bladder, stomach, small intestine, blood stream, and serous cavity were made and inoculation by feeding was also tried.

Direct inoculation into the gall-bladder, stomach, and small intestine and inoculation by feeding proved successful inasmuch as a certain percentage of the inoculated animals were found to harbor cholera vibrios in the alimentary canal. This was ascertained by bacteriologic examination of various parts of the digestive system, made in the great majority of cases immediately after death.

The intravesicular inoculation proved to be far superior to other methods. Practically every one of the animals inoculated in this way harbored cholera vibrios.

The duration of the condition, altho limited, appears to be sufficiently long for therapeutic experiments.