# MODEL OF A CHRONIC CHOLERA VIBRIO CARRIER STATE IN GERM-FREE RATS

Academician O. V. Baroyan,* O. V. Chakhava, I. N. Gailonskaya, N. Yu. Abrikosova, N. I. Blinova, and N. M. Shustrova

UDC 616-008.97-036.12-092.9

Germ-free or monofloral (contaminated with a nonpathogenic spore-bearing bacillus), and ordinary albino rats of the OFA strain were infected with Vibrio cholerae El-Tor of the "Ogawa" and "Inaba" serotypes ($6 \cdot 10^9$ bacterial cells in 1.5 ml physiological saline per rat). Approximately 1 week after infection the number of vibrios reached hundreds of millions per gram feces, and it remained at this level for over 100 days (period of observation). Newborn rats were infected naturally from the adult vibrio carriers and themselves became vibrio carriers. The number of vibrios excreted by animals which were germ-free and monofloral before infection was approximately the same. The cholera vibrios in the intestinal tract of ordinary (control) rats completely died and none were ever found in the feces.

KEY WORDS: cholera vibrio; germ-free animals; vibrio carrier state.

The ecology of the agents of intestinal infections, including cholera, is determined by their stay in the human body and in the external environment. It is not yet possible to state with confidence what is the relative importance of the influence of external environmental factors and of the human body on cholera vibrios. These questions are attracting the increasing attention of investigators because of the epidemiological features of present-day cholera. One of these features is the predominance of abortive forms and of a vibrio carrier-state, which play an important role in the spread of the infection [1-4]. Appropriate laboratory models are required for the experimental study of the principles governing the vibrio carrier state and for the development of methods of controlling it.

The object of this investigation was to create a model of a healthy vibrio carrier state. It was decided that germ-free animals would be the most suitable for such a purpose, for the normal microflora, which under certain conditions prevents the propagation of shigellas, salmonellas, cholera vibrios, and other enteropathogenic bacteria in the intestinal tract [8, 10-12], is absent in these animals.

## EXPERIMENTAL METHOD

The second, third, and fourth generations of germ-free OFA albino rats, propagated in plastic isolation cages in the germ-free laboratory of the Institute were used. Methods of growth and microbiological control of the sterility of the animals are described in textbooks of gnotobiology. The semisynthetic L474E12 diet was used [6, 7, 9]. OFA albino rats kept under the ordinary animal house conditions were used as the control.

The animals were infected with 6 strains of V. cholerae El-Tor, isolated from the external environment. To infect the animals a suspension of vibrios containing a mixture of 3 "Inaba" strains (373, 15093, 11836) and 3 "Ogawa" strains (281, 363, 15710) was prepared for infecting the animals. One of these mixtures was introduced into each isolation cage and poured into the drinking vessels. The total infecting dose was $6 \cdot 10^9$ bacterial cells in a volume of 1.5 ml per rat. During the 2 h before infection the rats received no water; they

*Academy of Medical Sciences of the USSR.

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 5, pp. 561-564, May, 1976. Original article submitted July 14, 1975.

TABLE 1. Cholera Vibrio Carrier State in Artificially and Naturally Infected Germ-Free Rats

| Group of animals before infection | No. of animals | Age on day of infection | Serotype of vibrios | Mean number of vibrios (in millions per gram feces) at different times (days) after infection | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 3 | 5 | 7 | 13 | 18 | 28 | 40 | 73 | 101 |
| Germ-free | 4 | 86 days | Ogawa | 0 | 3,22 | | 110 | 427 | 341 | killed | | — | — |
| Generation of germ-free rats born from vibrio carriers | 7 | Infected from vibrio carriers on eighth day after birth. Most vibrios found in contents of large intestine | | | | | | | | | | | |
| Monofloral | 5 | 1—13 months | Ogawa | single colonies | 500 | 16,5 | 625 | 642 | — | 185 | 357 | 280 | 345 |
| Generation of monofloral rats born from vibrio carriers | 9 | Infected from adult vibrio carriers. Vibrios found at autopsy on fifth day after birth in contents of large intestine | | | | | | | | | | | |
| Monofloral | 6 | 1—13 months | Inaba | 0,5 | 0,3 | 52 | 600 | 786 | — | 465 | 212 | — | 214 |
| Generation of monofloral rats born from vibrio carriers | 8 | Infected from vibrio carriers. Feces not tested before 25th day after birth | | | | | | | | 360 | 48 | | |
| Ordinary rats | 5 | 2 mos. | Ogawa | 0 | 0 | 0 | 0 | — | — | — | — | — | — |

therefore drank the above volume of suspension of cholera vibrios in 2-3 min.

The number of living cholera vibrios in the feces of the infected rats was determined by the usual method of serial dilutions and counting the number of colonies growing on alkaline agar.

## EXPERIMENTAL RESULTS

Five weeks before infection with cholera vibrios, germ-free rats were contaminated with a single species of nonpathogenic spore bearing aerobic bacteria. For decontamination, these animals were given antibiotics with their drinking water for 3 weeks. The antibiotics were then discontinued. Subsequent seedings of samples of feces for 1 week before infection with cholera vibrios were sterile. At that moment two groups of the animals were infected with cholera vibrios. These groups of animals are described in Table 1 as monofloral (before infection with the vibrios).

On the day after infection, vibrios were isolated from the fresh feces of the germ-free rats in a dilution of 1:1000. However, on the third day the number of vibrios in the feces fell appreciably, but later it rose again until the third to fifth day, when it reached a maximum of the order of $10^8$ bacterial cells per gram of feces. This number of cholera vibrios remained throughout the period of observation,i.e., until 100 days after oral infection. Meanwhile, from the third and seventh days after infection with vibrios, spore-bearing bacilli appeared again in the feces of these two groups of animals, not having been completely eliminated by the antibiotics used before infection with the vibrios. However, the number of spore-bearing bacilli remained lower than the number of vibrios (about two orders of magnitude lower).

Colonies of cholera vibrios isolated at different times after infection were indistinguishable from the original strains and had the typical morphology. Their enzyme activity likewise was the same as that of the strains used for infection.

Colonies of cholera vibrios grown from seedlings at different times after infection were indistinguishable from the original cultures and had the typical morphology. Their enzyme activity likewise was the same as that of the strains used for infection.

The agglutination test with Soviet and reference O-sera gave similar results as regards both titers and the serological identity of the strains of vibrios isolated. No change of serotype from Inaba to Ogawa or vice versa was observed.

No visible abnormalities in the state of health of the rats which were vibrio carriers occurred for almost 3.5 months after infection with vibrios. Toward the end of the third and ninth weeks after infection, one female in each group of animals gave birth to eight or nine rats and fed them all. The first bacteriological tests on the rats born in the isolation cage of the carriers of the Inaba serotype, undertaken on the 25th day after birth, showed that they were infected from their own parents naturally and had also become vibrio carriers. The number of vibrios from these animals starting from the 43rd day after birth and continuing until the end of the experiments, per gram of feces, was practically the same as in the case of artificially infected germ-free rats.

Germ-free rats which had never been decontaminated by antibiotics were used in the next experiment. They were infected by the method described above (orally) with a strain isolated from the other experimental rats on the 40th day of the carrier state of vibrios of the Ogawa serotype. On the sixth day of the experiments one of the females monoassociated with vibrios gave birth to seven young rats. These young animals were killed and autopsied for bacteriological investigation of the contents of the small and large intestine. Many vibrios were isolated from these rats on the eighth day after birth. In the first 2 days, single colonies of vibrios of the Ogawa serotype were grown from seedings of the feces of the adult animals (germ-free before infection with vibrios) in a dilution of 1:100. On the third day after infection the number of vibrios per gram of feces was $3.22 \cdot 10^6$, and on the subsequent days it reached the same level as in the first two groups of animals described previously. Hence, it follows that the presence of a monoflora of nonpathogenic spore-bearing bacteria in the intestine did not affect the number of cholera vibrios in the intestine of the germ-free animals.

On the 18th day after infection, rats monoassociated with cholera vibrios (germ-free before infection) were killed for histological examination, together with some vibrio carriers from the other groups.

The number of vibrios in the feces of the germ-free rats was thus several orders of magnitude greater than in the formed stool of human vibrio carriers. The consistency of the fecal masses of the germ-free vibrio carriers, including those monoassociated with cholera vibrios, was the same as in the germ-free rats.

As a rule the vibrio carrier state in man is relatively short in duration, but in a small percentage of cases it may be very prolonged. In addition, a healthy vibrio carrier state arises by no means invariably after infection with cholera [5]. It is shown in the paper cited above that 100% of orally infected adult rats and of young rats in contact with vibrio carriers became chronic carriers of the agent of cholera.

The results described above are in agreement with those obtained by Miller et al. [10, 11] as regards the possible creation of a prolonged vibrio carrier state in NIH mice germ-free before infection. In the investigation now described we have not only developed a model of a chronic vibrio carrier state in germ-free and monofloral rats, but also shown that natural transmission of the agent of cholera is possible from the carrier to healthy animals, which in turn become vibrio carriers in the same way.

## LITERATURE CITED


1. O. V. Baroyan, Recent Data on the Epidemiology and Prophylaxis of El-Tor Cholera [in Russian], Moscow (1967).
2. O. V. Baroyan, El-Tor Cholera [in Russian], Moscow (1971).
3. P. N. Burgasov, El-Tor Cholera [in Russian], Moscow (1971).
4. N. N. Zhukov-Verezhnikov and E. P. Kovaleva, Current Problems in the Theory and Practice of Anti-cholera Measures [in Russian], Kiev (1971).
5. T. S. Kereselidze, International Aspects of the Control of Cholera [in Russian], Moscow (1971).
6. O. V. Chakhava, Gnotobiology [in Russian], Moscow (1972).
7. M. E. Coates (editor), Germ-Free Animals in Research, London (1968).
8. B. Greenberg, in: Symposia of the 9th International Congress of Microbiologists [in Russian], Moscow (1966), p. 269.
9. T. D. Luckey, Germ-Free Life and Gnotobiology, New York (1963).
10. C. E. Miller and R. B. Sack, in: Advances in Germ-Free Research and Gnotobiology (International Symposium) (ed. by M. Miyakawa and T. D. Luckey), Cleveland, Ohio (1968), p. 302.