ON THE METHODOLOGY OF OBTAINING EXPERIMENTAL PARATYPHOID INFECTION IN RABBITS*

F. Kh. Kucheryavy

From the S. M. Kirov Leningrad State Order of Lenin Institute for the Development of Physicians (Director: Professor N. L. Blinov) and the Department of General Pathology (Head: Corresponding Member of the Academy of Medical Sciences of the USSR Professor P. N. Veselkin)

(Received December 7, 1955. Submitted by Acting Member of the Academy of Medical Sciences of the USSR N. N. Anichkov)

It is known from the experiments of A. A. Valdman that rabbits are quite susceptible to bacteria of the Typhoid-paratyphoid group and that under certain infective conditions they present a picture of the disease which is close in its course to the pathomorphological picture of paratyphoid in man. However, until recently it has not been possible to produce paratyphoid in rabbits by the process of natural infection—through the mouth, because their stomach is a barrier, barring the penetration of live virulent microorganisms into the duodenum [1, 2, 3, 4, 5, 8].

According to the data of many authors [6, 7], the gastric juice of rabbits is very acid and has great powers of digestion; in addition, the evacuation of food masses from the stomach proceeds extremely slowly. Therefore the attempt to infect rabbits by mouth with even massive doses (undiluted boullion culture) of Bact. typhi murium (Breslau) by feeding or by administering it into the stomach through a tube does not cause the disease.

Taking into account the peculiarities of the digestive tract of the rabbit, A. A. Valdman in 1930 suggested an operative method of infection which consists of introducing the culture by means of a syringe directly into the lumen of the duodenum after cutting (under narcosis) the abdominal wall. The author studied the pathologico-anatomical picture of the incipient disease and established the similarity of the morphological changes in rabbit paratyphoid with the typhoid form of paratyphoid infection in man.

The indicated method of achieving paratyphoid infection has a number of favorable aspects due to the standardization of the conditions of infection and to the exact dosage of the culture of microorganisms which reaches the intestinal tract.

However, the operative method of A. A. Valdman, aside from some technical difficulties, is unavoidably involved with side effects on the reactivity of the system at the moment of infection and does not permit investigation of the pure form of the incubation and initial stages of the disease, which are especially important in studying the pathophysiology of the process.

Taking into account all the above, we set as our problem the achievement of paratyphoid infection by infecting rabbits through the mouth.

Our experiments showed that it is possible to obtain typical paratyphoid disease in rabbits both by administering a culture of Breslau bacteria into the stomach with bile or milk through a tube and also when the rabbit drinks a mixture of milk with the culture. Bile and milk, as is known, are a good culture medium for bacteria of

* Presented at the Conference of Young Scientists of the S. M. Kirov Leningrad State Order of Lenin Institute for the Development of Physicians, June, 1954.

the typhoid-paratyphoid group; at the same time, bile decreases the digestive effect of the gastric juice, depressing gastric secretion and neutralizing the acidity of the gastric juice. In using milk the possibility is not excluded that sufficiently virulent microorganisms will be preserved in the casein lumps which are formed when milk curdles, and that the microbes will go on into the duodenum with the first portions of the administered liquid, which pass quickly through the stomach.

In the first series of experiments we introduced 5.0-10.0 ml of beef bile (undiluted or diluted to 1:3, 1:5) through a tube into the stomach. Then culture was administered in doses of from 15 to 100 million bacteria, followed by 5.0-10.0 ml of bile. In the second series of experiments, the culture in a dosage of 500 million mixed with 100-130 ml of milk was introduced into the stomach through a tube.

In the third series of experiments a method of infection which is even closer to natural conditions was tested. The rabbits were allowed to drink freely from the feeder 100-130 ml of milk to which 500 (sometimes 250) million bacteria of a 24-hour culture of the Breslau bacillus grown on meat agar had been added. This amount of milk was usually drunk by the animal at once, sometimes in several portions in the course of 10-20 minutes. Rabbits which were transferred to dry feed without water on the day before the experiment drank the milk especially greedily. If the animal did not drink all the milk, the remainder was administered through a tube. At first we did not feed the animal on the day of infection, but later we fed during the entire experiment. After infection, the animals were subjected to thermometry every 2-3 hours around the clock, daily weight determination, recording of the general behavior, mobility, nature of the stool, acceptance of food. In part of the experiments the gaseous exchange (by V. V. Pashutin's method) was determined. The animals which did not succumb were killed on the 8, 12, 14th day after infection usually. All the rabbits were subjected to autopsy and the nature and extent of the pathological changes in the lymphatic apparatus of the intestinal tract and other organs were noted. During autopsy we made cultures from the blood and organs on Endo's differential medium and took pieces of the organs for subsequent histological examination. In all, 74 rabbits were infected.

In all cases of infecting the rabbits by mouth, a typical picture of paratyphoid infection was obtained. The severity of the disease varied depending on the dose of the microorganisms administered and the conditions of infection (bile or milk and their amount). Thus, when infection was with bile through a tube with relatively small doses of the Breslau bacillus (from 15 to 100 million bacteria + 10-20 ml bile) light forms of the disease were obtained in 22 rabbits with a long incubation period (5-7 days), with slight fever or even without it. In all cases the disease was confirmed by bacteriological and pathomorphological investigation.

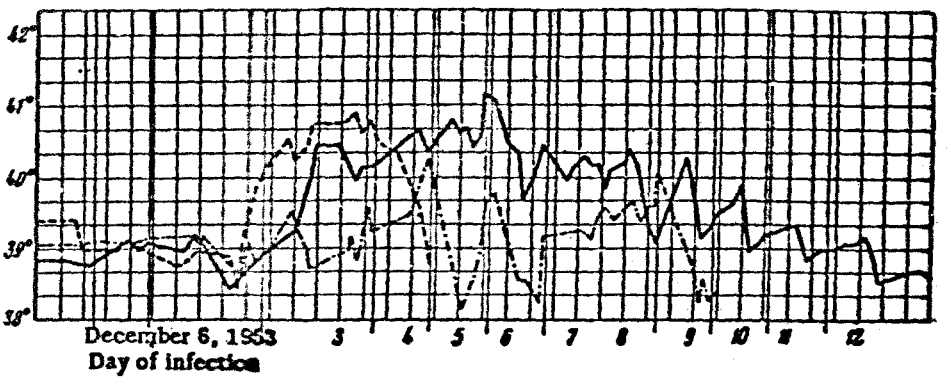

Fig. 1. Examples of temperature curves of rabbits with paratyphoid infection.

* In November, 1954, I. P. Vaitkus reported on the light forms of the disease which he obtained by introducing large doses of Breslau bacteria (220 billion microbes) mixed in 100 ml of physiological solution into the stomach of rabbits. For this reason the dilution of the gastric juice by the administered liquid may be believed to have a certain significance when milk is administered also.

However, when bile is used it is impossible to be certain that the bile itself has no effect on the development of the disease. Therefore it seemed more perceptive to infect the rabbits by mouth with a culture of Bact. typhi murium mixed with milk.

In experiments of this group, the rabbits independently drank a mixture of the culture (500 million bacteria) with 100-130 ml of milk. All of them became diseased after a short incubation period (2-3 days). In the great majority of cases typical, serious forms of the disease developed with fatal outcome.

In later work using this method, we infected 39 more rabbits and 16 rabbits were infected in a similar way through a tube. The development of the disease in these animals did not differ from that of the experimental group described below.

In the majority of cases of this group (25 out of 32) the severity of the morphological changes in general corresponded to the severity of the clinical course of the disease. However, in the remaining 7 cases, this correspondence was not observed. The disease, as a rule, was accompanied by a clearly evident fever which lasted an average of 4-5 days (see table). Examples of the temperature curves are shown in Figure 1.

Course of Paratyphoid and Outcome of the Disease in Rabbits Infected by Mouth with a Culture of the Breslau Bacillus (500 million bacteria) Mixed with Milk

Number of infected rabbits	Day after infection on which temperature rise occurred				Day after infection on which death occurred			Total dead	Survived
	1	2-3	4-5		1-5	6-10	>10		
32	3	22	6	1[1]	10	11	1	22	10

[1] No temperature rise was observed.

Autopsy of the animals which died or were killed at various times in the lymphatic system of the intestinal tract was revealed a typical picture of destruction which corresponded, in general, to that described in the investigations of A. A. Valdman with the intraduodenal method of infection.

Fig. 2. Multitudinous necrotic knots in the lymphatic follicles of Peyer's patches in the small intestine and appendix.

Even from the 2-3rd day after infection, autopsy disclosed swollen lymphatic structures in the intestines. From the 3-4th day after infection small knots of a yellowish-white color appeared, which later enlarged. These knots corresponded to the necrotic areas in the region of the lymphatic follicles (Fig. 2). The necrotic areas, later, enlarging, sometimes joined and acquired the form of solid necrosis in serious forms of the disease. Frequently ulcers were formed where the necrotic masses separated away. They could be small, multitudinous, but more often they were single, large, with uneven edges, with decomposition in the center, covered by a dirty gray, sometimes yellow, film.

Histological investigation of the lymphatic formations in the intestines, which was carried out by us in all cases, gave results similar to those described by A. A. Valdman with the operative method of infection.

Thus, rabbits infected by our method develop a disease which is basically analogous in its clinical form as in its pathological-anatomical changes to that of Valdman's experiments, i.e., the result of operative infection.

LITERATURE CITED

[1] A. A. Valdman, Arkh. Biol. Nauk. 31, 1, 17-23 (1931).

[2] A. A. Valdman, Arkh. Biol. Nauk 53, 1, 146-157 (1939).

[3] A. A. Valdman, Arkh. Biol. Nauk 55, 1, 163-175 (1939).

[4] A. A. Valdman, Vestnik Akad. Med. Nauk 1951, No. 3, 31-37.

[5] F. Kh. Kucheryavy, Effect of Artificially Increasing and Lowering the Body Temperature on the Development of Morphological Changes, the Course and the Outcome of Experimental Paratyphoid in Rabbits, Author's abstract of dissertation,* (Leningrad, 1955).

[6] A. Sineshchekov, T. Poberezhskaya, and N. Savranskaya, Fiziol. Zhur. SSSR 27, 1, 92-94 (1939).

[7] I. A. Troitsky, Problemy Zhivotnovodstva 1937, No. 8, 152.

[8] A. I. Ugleva, Experimental Paratyphoid Infection Caused by Salmonella Type C, Author's abstract of dissertation,* (Leningrad, 1953).

* In Russian.