THE USE OF THE REACTION OF INCREASE OF PHAGE TITER IN THE DETECTION OF FLEXNER DYSENTERY BACTERIA IN INFECTED RABBITS

A. Iu. Iliutovich, Z. S. Petrova, E. E. Golubeva, and R. S. Chetvervina

The Stavropol' Institute of Vaccines and Sera (Head — Candidate of Medical Sciences, V. M. Kruglikov)

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The spread of dysentery bacteria throughout the human body is of considerable interest from the point of view of solving problems of the pathogenesis and also of specific prophylaxis of dysentery. The views expressed in the literature on the spread of dysentery microorganisms in the body are based on bacteriological detection of the agent in the blood or in organs of persons dying from dysentery [3, 4], and on bacteriological examinations of the blood and bile during life (Denner, Kulesha, Besredka et al.).

In recent years series of experiments have been performed to study the pathogenesis by means of radioactive isotopes. V. I. Ivanov and his co-workers [2] describe their findings of the distribution in animals of antigens of typhoid bacteria containing radioactive phosphorus $^32$P. V. L. Troitskii and his co-workers [8], using Flexner-Sonne dysentery vaccine labeled with radiophosphorus, demonstrated rapid penetration of microorganisms into the blood stream after infection of rabbits per os. The results so far obtained suggest that other methods of indication of dysentery microorganisms in the body are necessary in order to elucidate the problems of pathogenesis and immunity in dysentery.

In the present paper we describe an experimental study of the possibility of using the reaction of increase of phage titer (the method of V. D. Timakov and D. M. Gol’dfarb) to detect dysentery microorganisms in infected rabbits. This reaction was widely tested by these authors for diagnostic purposes in detecting dysentery bacteria in the stools of patients and carriers, and also in various environmental objects (in water, and washings from the hands and various utensils).

EXPERIMENTAL METHOD

The principle of the reaction is based on the increase in the titer of highly sensitive indicator phage during its interaction with dysentery bacteria contained in the material for examination. It enables these microorganisms to be found in a short time (10-20 hrs) in very low concentrations. The experiment was performed on 53 rabbits, divided into 4 groups. The animals were infected with a mixture of dysentery strains of Flexner a, b and c types, whose virulence, expressed $LD_{100}$, was equal to 500 million bacterial bodies per 1 ml. For infection we used washings in physiological saline of 24-hr cultures with an optical standard of 1 and $4 \cdot 10^9$ bacterial bodies per 1 ml.
The animals were infected by groups - intravenously, subcutaneously and orally, mainly with the $1 \cdot 10^9$ suspension. In the group of animals infected orally the experiment was repeated with the $4 \cdot 10^9$ suspension (4th group). In order to confirm the reliability of the results in each group there were control rabbits. The infected animals were killed at various intervals – 1, 3, 6, 24, 48 and 72 hrs and 5, 15 and 30 days after administration of the Infective material. The following organs were investigated: liver, spleen, kidney, lungs, lymphatic glands, mucous membrane of the small and large intestine, ascending and descending colon, bone marrow and brain.

In the specimens obtained the presence of dysentery bacteria was determined by the reaction of Increase of phage titer* and by a bacteriological method. For the RIP we used indicator phage obtained from the Central Institute of Epidemiology and Microbiology.

The RIP was estimated by the Increase in the number of patches (sterile areas). An Increase in the number of patches of 3-5 times that of the control phage in a degree of $10^{-8}$ was regarded as a + reaction, from 5-7 times as ++, from 7-10 times as +++ and over 10 times as ++++.

**EXPERIMENTAL RESULTS**

Altogether we performed 90 analyses of organs from rabbits infected intravenously (9 animals, excluding controls). In the experiments on organs of animals examined 1 and 3 hrs after infection the results of the RIP and bacteriological investigations agreed. It was found that at 1 hr after infection a culture was found by both methods in the liver, spleen, bone marrow, lung, lymphatic glands and kidney; after 3 hrs, in addition to the organs just mentioned it was found in the descending colon and the brain. After 6 hrs we obtained a positive result in all the organs examined except the lymphatic glands and the ascending and descending colon, where bacteria were discovered only by means of the RIP, cultures giving negative results (Table 1).

The changes in the discoveries of the microorganisms in the subsequent hours and days show that until 3 days the almost complete dissemination of microorganisms in the examined organs persists; later they begin to disappear and on the 30th day after infection they can no longer be detected. In some cases, as seen from Table 1, the agent could be found only by means of the RIP, in particular in the mucosa of the large intestine, from 3 hrs after infection until the 15th day. The quantitative changes in the microorganisms in the intestinal mucosa are well reflected by the intensity of the RIP, which weakens sharply at 48 hrs.

The results of this series of experiments showed that in 62 positive analyses the results of the RIP and the bacteriological method agreed in 52 cases (83.8%); 10 analyses, or 16%, gave a positive RIP along with a negative bacteriological examination. The use of the RIP along with the bacteriological method in this case showed that after infection of rabbits by the intravenous method the agent could be detected in the mucosa of the colon only 3 hrs after infection, and persisted stubbornly in all parts of the intestine for 5 days, while it could be observed in the ascending colon on the 15th day after infection.

Nine animals were infected subcutaneously (1 ml of $1 \cdot 10^9$ bacterial suspension). The material was injected into the middle of the abdomen. The rabbits were killed and examined on the same days and hours. Microorganisms were found by both methods after 48 and 72 hours (Table 2) in the ascending colon. On the 5th day they were found only by means of the RIP in the following organs: lymphatic glands, small intestine, ascending and descending colon, bone marrow. In subsequent days (15th and 30th days) all the analyses were negative (see Table 2).

The subcutaneous infection experiment also demonstrated the selective power of the dysentery organisms to become localized in the mucosa of the small and large intestine. In addition the greater sensitivity of the RIP in comparison with the bacteriological method of investigation was established.

The 3rd and 4th groups, each of 9 rabbits, were infected orally with 1 and $4 \cdot 10^9$ suspensions of bacteria. In the 3rd group positive bacteriological examination and RIP results were obtained with lung tissue 1 and 3 hrs after infection. In addition a positive culture and negative RIP were found in the bone marrow after 1 and 48 hrs. A positive RIP alone was also found in the lymphatic glands and the mucosa of the descending colon after 48 hrs. At this time the positive results by both methods were in agreement for cultures isolated from the mucosa of the descending colon.

* Abbreviated RIP in text.