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# STYBILITY OF THE TOXIC AND SEROLOGICAL PROPERTIES OF K. COLI ENDOTOXIN IN THE INTESTINAL TRACT OF RATS

By

I. BERCZI, T. BEREZNAY and L. BERTOK

Velerinary Research Institute of the Hungarian Academy of Sciences, PHYLAXIA State Serum Institute and "Frederic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary

toxic effect could be induced by a large dose of perorally administered endotoxin in rats. No toxic effect was experienced even in animals hypersensitized to endotoxin by lead acetate and treated with 48/80 or X-irradiated to damage the intestinal mucosa [2]. The problem arises whether endotoxin gets detoxified in the gastrointestinal tract by enzymes or in sunK' oilier way and that would acount for the failure of the absorbtion experiments.

Female rats weighing 100 g (90-110 g) or 150 g (140-150 g) and 10 days old chick embryos were used. Endotoxin was prepared in this labo-**mIhiv** I'^ the warm phenol-water-method [4] from the fermentor culture of I ho I'l. coli 089 strain. Immune sera were produced in rabbits by the E. coli 089 endotoxin-easeine complex [1]. The contents of the gastro-inleslinal tracts were prepared in the following manner. The complete giislro-intestinal content of five rats forming an experimental group was dilulod I o 500 ml, mixed for an hour by a magnetic mixer and centrifuged. Corresponding to endotoxin extraction, 50 ml from the supernatant were Irealed with 50ml of 90 per cent phenol. Following dialysis, the suspension obtained was used for toxicity and serological test. To obtain liver and spleen on preparations, 25 per cent organ homogenates were prepared from both the liver and the spleen of each animal. Next, the extracts were prepared by phonol, in the usual manner. Serological tests were performed by haem-agglutination-'-inhibition techniques. (The sensitivity of the haemagglutinalion system was 0.4 [xg/ml.) The toxicity of the single preparations was sludied on rats hypersensitized by lead acetate (Fisher Sci. Co. N.J., USA) on the one hand (5mg/100g, i.v.) [3], and on 10 days old chick embryos, on (ho other [1].

The ani of 20 etherized rats were closed by sutures. Next, each animal en 50 mg endotoxin by a gastric tube. Five animals were killed

immediately, further groups of 5 animals after 6, 12 and 24 hours, respectively. The intestinal contents were pooled and prepared by groups. Individual preparations were made from both the liver and spleen of the animals killed in the 24th hour.

"Negative-control" preparations were made from the gastrointestinal contents, spleen and liver of five untreated rats. "Positive-control" preparations were obtained from the liver and spleen of five rats, one hour after the intravenous injection of 10 mg endotoxin and also from the same organs of further five rats one hour after the intravenous injection of 100 u.g endotoxin.

The results of the toxicity test of the gastrointestinal preparations are shown in *Tables I* and *II.*

The data of both *Tables (I* and *II)* prove that endotoxin does not lose its toxic properties in the gastrointestinal tract of rats even for after 24 hours.

*Table I*

**Stability of the toxic properties of E. coli endotoxin in the gastrointestinal tract of rats (in vitro) Experiment with lead acetate liypersensitized rats**

| Dilution of preparation* | Death ratio: died/total | | | | |
|---|---|---|---|---|---|
| | Normal preparation | 0 | 6h | 12h | 24h |
| concentrated | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| 10 x | 0/5 | 5/5 | 5/5 | 4/5 | 5/5 |
| 100 x | 0/5 | 5/5 | 1/5 | 5/5 | 3/5 |
| 1000 x | 0/5 | 2/5 | 1/5 | 3/5 | 1/5 |

*** = Phenol-water extracts of the gastrointestinal contents of animals killed 0, 6, 12 and 24 hours, respectively, after the administration of 50 mg endotoxin by gastric tube.**

*Table II*

**Stability of the toxic effect of E. coli endotoxin in the gastrointestinal tract of rats (in vitro)* Experiments on 10 days old cliiclt embryos**

| Dilution of preparation* | Death ratio: died/totai | | | | |
|---|---|---|---|---|---|
| | Normal preparation | 0h | 6h | 12h | 24h |
| concentrated | 2/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| 10 X | 0/5 | 5/5 | 5/5 | 5/5 | 5)5 |
| 100 x | 0/5 | 0/5 | 1/5 | 3/5 | 1/5 |
| 1000 x | 0/5 | 2/5 | 0/5 | 0/5 | 0/5 |

*** = Phenol-water extracts of the gastrointestinal contents of animals killed 0, 6, 12 and 24 hours, respectively, after the administration of 50 mg endotoxin by gastric tube.**

The indirect haemagglutination—inhibition reaction was used to test the single preparations for the presence of endotoxin content. It could be established that endotoxin retains also its serological activity in the gastrointestinal tract of rats.

The organ preparations were also tested serologically for the presence of endotoxin. It has been established that only those organ preparations inhibit in the system (contain endotoxin) which had been obtained from the organs of rats inoculated with endotoxin. The inhibition was proportional to the injected dose of endotoxin. No endotoxin (no inhibition) could be demonstrated by our serological methods in the organ preparations from rats treated with endotoxin perorally.

The liver preparations were tested also for toxicity by inoculating them

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into 10 days old chick embryos. It has been established that not only rats given endotoxin parenterally but also the liver preparations from "negative-control" rats are toxic to 10 days old chick embryos. Although the "positive-control" liver preparations proved to be somewhat more toxic, the differences were not significant.

Summarizing the results of the test, it might be established that perorally administered endotoxin retains both its toxic and serological effects in the gastrointestinal tract of rats. Accordingly, negative experimental results on endotoxin absorption cannot be interpreted by the destruction of endotoxin in the gastroinstestinal tract, much more by the absorption failure of endotoxin. The serological examination of the organ preparations also supports this assumption. Twenty-four hours after the peroral administration of 50 mg of endotoxin no unabiguously positive result was obtained in any of the cases, while the organ preparations reacted positively to the intravenous administration of 100 [j.g endotoxin. In course of other experiments it has also been experienced that also normal liver preparations are toxic to 10 days old chick embryos. Although the toxicity of the "positive" organ preparations is somewhat higher than that of the normal, there is no appreciable difference between the two groups, in spite of the significant differences showing in their haemagglutination—inhibitions. Maybe that the serologically active groups of endotoxin are not responsible for the toxic effect.

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