ANNALES
IMMUNOLOGIAE HUNGARICAE

11—12.

1968

EXPERIMENTS TO INDUCE ENDOTOXINTOLERANCE AND TOXIC EFFECTS BY PERORAIXY ADMINISTERED E. COLI ENDOTOXIN IN RATS

By

I. BERCZI, L. BERTÓK, K. BAINTNER and B. VERESS

Veterinary Research Institute of the Hungarian Academy of Sciences, '.'Frederic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, Research Institute for Animal Physiology and Ilnd Department of Pathology, University Medical School, Budapest, Hungary

One of the most important problems raised in connection with the pathomechanism underlying the clinical pictures induced by endotoxins from gram-negative bacteria is that of endotoxin absorption from the intestines : whether endotoxin is absorbed through the undamaged intestinal mucosa of undisturbed functioning or, inversely whether absorption may occur through damaged intestinal mucosa.

Earlier experiments on endotoxin absorption gave contradictory results. According to a number of authors, though to an ever varying extent, endotoxin is continously absorbed from the intestinal tract [7, 12, 16, 17,18, 20, 21, 24]. On the other hand, other authors could not demonstrate "endotoxin absorption [4, 9, 11, 13, 19, 25].

In former experiments no toxic effect could be elicited by a large dose of perorally administered endotoxin even in newborn animals, though macro-molecules (cholostrum proteins) are known to be resorbed from their intestinal tract [1]. In an attempt to promote endotoxin absorption the intestinal mucosa of the animals was damaged in the experiments reported on here. Also, more sensitive methods than earlier were used to demonstrate endotoxin absorption.

The histamine liberator compound 48/80 (Burrows Wellcome Lab. Canada) [5] or X~irradiation [14] were used to damage the intestinal mucosa. To be able to demonstrate the slight dose of endotoxin, the female rats of 150 g (140-160 g) had been hypersensitized by lead acetate (Fisher Sci. Co. N.J. USA) [22]. Endotoxin was prepared in this laboratory by the warm phenol-water method [23] from the fermentor culture of the E. coli 089 strain. Each animal was subjected to whole body irradiation separately (170 kV, 10 mA, 0.5 mm Cu filter without tube, 30 cm FSD, 750 r air dose). Rats were observed for 30 days after irradiation. To anticipate any inter-

current infection, the drinking water the animals were given contained penicillin-streptomycin. After killing the animals, their organs were removed, fixed immediately in buffered formalin for histological examinations and processed the usual way. The sections were stained with haematoxylin-eosin. The first part of the experimental series was designed to reveal whether endotoxin tolerance may be elicited by perorally administered endotoxin. The experimental scheme and the results are shown in *Table I.*

Table I

Experiment to induce endotoxin—tolerance by perorally administered endotoxin in rats

Group	Pretreatment	Challenge	Death ratio died/total
1	—	Endotoxin	1/5
2	10 mg endotoxin per os every 2nd day, 4 times	2.5 mg i.v.	1/5
3	20 μg endotoxin i.p. every 2nd day, 4 times		0/5
4	—	Endotoxin	2/5
5	10 mg endotoxin per os every 2nd day, 4 times	5.0 mg i.v.	3/5
6	20 μg endotoxin i.p. every 2nd day, 4 times		1/5
7	—	Endotoxin	5/5
8	10 mg endotoxin per os every 2nd day, 4 times	7.5 mg i.v.	4/5
9	20 μg endotoxin i.p. every second day, 4 times		1/5
10	—	Endotoxin	5/5
11	10 mg endotoxin per os every second day, 4 times	10.0 mg i.v.	5/5
12	20 μg endotoxin i.p. every second day, 4 tim		3/5

As it appears from the *Table I.* there is no difference between the endotoxin sensitivities of the untreated groups (1, 4, 7, 10) and those treated with endotoxin pcrorally (2, 5, 8, 11). Naturally, endotoxin tolerance has not failed to develop in the majority of animals treated with endotoxin intraperitoneally (3, 6, 9, 12) for the sake of comparison. It may be concluded that no endotoxin tolerance may be elicited in rats by endotoxin administered perorally.

The second part of the experimental series was designed to clear up whether toxic effect may be elicited by perorally administered endotoxin in rats with intestinal mucosa damaged by 48/80 and hypersensitized by lead acetate. According to informative experiments no \xg of endotoxin si absorbed from the intestinal tract of undamaged control animals, since not

even the per os administration of 10 mg caused appreciable death among rats hypersensitized by lead acetate. One to three jj.g of intravenously injected endotoxin induces lethal shock in rats pretreated with lead acetate 48/80 was used in an attempt to damage the intestinal mucosa. As established by informative experiments, as early as 6 hours after the intravenous administration of 150 |zg of 48/80 (dissolved in 0.5 ml distilled water), histologically well definable, characteristic lesions develop in the intestines.

Table 11

Experiment to induce toxic effect by perorally administered endotoxin in rats pretreated with 48/80 and hypersensitized by lead acetate

Group	Critical period of treatment			Death ratio died/total
	0h 48/80	6h endotoxin	7h lead acetate	
1	150 μg i.v.	—	—	0/5
2	—	—	5 mg i.v.	0/5
3	150 μg i.v.	—	5 mg i.v.	0/5
4	150 μg i.v.	3 μg i.v.	5 mg i.v.	5/5
5	150 μg i.v.	10 mg p. os	5 mg i.v.	0/5

Accordingly, endotoxin was administered 6 hours after treatment with. 48/80 and lead acetate, designed to demonstrate incidental absorption, in the 7th hour. The scheme and results are shown in *Table II.*

As it may be seen from *Table II.* not even in rats pretreated with. 48/80, i.e. having a damaged intestinal mucosa, is so much (2-3 *\tg)* of the-pcrorally administered 10 mg endotoxin resorbed that would be sufficient to elicit a lethal shock in the organism hypersensitized by lead acetate,, as do 3 **[Ag** of endotoxin injected intravenously.

In the third part of the experimental series the effect of whole body irradiation with 750 r on the absorption of endotoxin introduced into the intestinal tract was studied. Previous histological study confirmed the damaging effect of irradiation on the intestinal mucosa.

In agreement with literature [15] it has been established that after whole body irradiation with 750 r appreciable lesions develop in the mucous-membrane of the intestines 48 to 96 hours after irradiation. Accordingly, some animals were treated per os and others, for the sake of control parenterally, in that time period. The experimental scheme and the results are-shown in *Table III.*

As it may be seen from *Table III.* no large amount of per os administered endotoxin will be absorbed from the intestinal tract of X-irradiated animals. On the other hand the small dose of endotoxin administered for the sake of comparison parenterally (intra-peritoneally) produced 100 per cent lethality. The few death cases observed in the irradiated and per os endotoxin treated groups all occurred but later, during the 30 days observation period, and may be considered as the result of irradiation. Namely,.

the same death rate (2 animals) occurred in the only irradiated, controi group [1], too.

in the fourth part of the experiments, the original scheme was completed only inasmuch as, in addition to irradiation, lead acetate was also-given to the rats at the appropriate date. This was meant to favour the; demonstration of any incidental slight endotoxin absorption. However,' as regards endotoxin absorption, also this experiment was unsuccessful.^

Table III

Experiment to induce toxic effect by perorally administered endotoxin in rats pretreated with X-irradiation

Group	Treatment		Death ratio died/total (in 30 days)
	X-ray dose	Endotoxin	
1	750 r	—	2/10
2	750 r	20 mg i.p. 48h after irradiation	10/10
3	750 r	100 mg p. os 48h after irradiation	2/10
4	750 r	20 mg i.p. 96h after irradiation	10/10
5	750 r	100 mg p. os 96h after irradiation	1/10

Summarizing, it may be established that neither endotoxin tolerance nor any toxic effect could be elicited in rats by perorally administered endotoxin even the 500 to 3,000 fold of the dose known to be lethal if administered parenterally (i.v. or i.p.). This was the case also with rats with intestinal mucosa previously damaged either with 48/80 or with X-irradiation. It may be concluded that under our experimental conditions no endotoxin is absorbed from the intestinal tract, or it did so to such a slight extent, which could not be demonstrated by our methods.

REFERENCES

1. *Bérezi, I., Baintner, K. & Antal, T.:* Zbl. Vet.-Med. Reihe B, 23:570, 1966.
2. *Bloom, W. & Bloom, N, A.:* Histological Changes after Irradiation, in "Radiation Biology" (Hollaender, A. ed.) Vol. 1. McGraw-Hill Book. Co. New York, 1954.
3. *Braun, H.:* Exp. Cell Res. 20:267, 1960.
4. *Culbertson, W. B., Elstun, W., Cole, W. & Altemeier, W. A.:* A.M.A. Arch. Surg. 79:185, 1959.
5. *Fell, B. F., Boyne, R. & Cuthbertson, D. P.:* J. Path. Bact. 80:445, 1961.
6. *Friedman, N. B.;* J. Exp. Med. *81:553,* 1945.
7. *Greene, R., Wiznitzer, T., Rutenburg, S., Frank, E, & Fine,* J.; Proc. Soc. Exp. Biol. Med. 205:261, 1961.
8. *Hampton, J. C.* & *Quastler, H.:* IVth Internatl. Cong, of Electronmicroscopy, 2:480, 1960.
9. *Hardy, E. G., Morris, G. C, Jow, E. M., Haynes, B. W. & DeBakey, M. E.:* Ann. Surg. *139:282,* 1954.

10. *Matthias, D., Stetlmacher, W. & Baumann, G.:* Mh. Vet.-Med. 21:667, 1966.
11. *McCluskey, R. T., Zweifach, B. W., Antropol, W., Benacerraf, B. & Nagler, A, L.:* Am. J. Path. 57:245, 1960.
12. *Miler, I., Kostka, J., Simek, L. & Lane, A.:* Folia Microbiol. 0:277, 1964.
13. *Parant, F., Parant, M., Charlier, H., Sacquet, E. & Chedid, L.:* Ann. Inst. Pasteur, 120:198, 1966.
14. *Pierce, M.:* The gastrointestinal tract, in: "Histopathology of Irradiation" (Bloom, "W. ed.) McGraw-Hill Book. Co., New York, 1948.
15. *Quasiler, H.:* Radiology, 73:161, 1959.
16. *Ravin, H. A. & Fine, J.:* Fed. Proc. 22:05, 1962.
17. *Ravin, H. A., Rutenburg, S. H. & Fine, J.:* Proc. Soc. Exp. Biol. Med. 97:436, 1958.
18. *Ravin, H. A.,'Rowley, £>., Jenkins, C. & Fine, J.:* J. exp. med. *112:783,* 1960.
19. *Sanford, J. P. & Noyes, H. E.:* J. Clin. Invest. 37:1425, 1958.
20. *Schweinburg, F. B. & Fine, J.:* J. Exp. Med. 112:793, I960.
21. *Schweinburg, F. B.,Shapiro,P. B., Frank, E. D. & Fine, J.:* Proc. Soc. Exp. Biol. Med. 05:646, 1957.
22. *Selge, B., Tuchweber, B. & Bertók, L.:* J. Bact. £2:884, 1966.
23. *Westphal, 0., Laderitz, O. & Bister, F.:* Z. Naturforsch. *7b:U8,* 1952.
24. *Wiznitzer, T., Schweinburg, F. B., Atkins, N.* & *Fine, J.:* J. Exp. Med. 212:1167, 1960.
25. *Zweifach, B. W.:* Ann. N.Y. Acad. Sci. 75:313, 1961.