

## Temperature Factors in the Action of Certain Bacterial Endotoxins.

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It has been previously reported that the endotoxin of *Shigella paradysenteriae* (Flexner) administered intraperitoneally to gravid rabbits induces a hyperthermia which develops at the same time as decidua-placental hemorrhage.<sup>1</sup> The same endotoxin when injected into mice induces hemorrhage both in implanted tumors and in decidua-placental tissues.<sup>2</sup> The concurrence of thermal and histopathological effects in the rabbit following injection of the Flexner endotoxin suggested a study of this relationship in the mouse.

**Experimental.** Record was kept of rectal temperatures following the intraperitoneal injection of the endotoxin-containing acetone precipitate of the residue of dialyzed whole cultures of *Shigella paradysenteriae* (Flexner) into (1) mice bearing 7-day-old implants of mouse sarcoma 180, and into (2) mice at approximately the 15th day of pregnancy.\* Endotoxin-containing extracts of *Salmonella typhimurium* and *Rhodospirillum rubrum* were similarly employed. These 3 organisms, 2 closely related and the third taxonomically remote from the other 2, were chosen as representative gram-negative bacteria. Methods of culture preparation and standardization of these materials are described elsewhere.<sup>3</sup> An

arbitrary dose of  $\frac{1}{4}$  LD<sub>50</sub> was selected for testing the 3 preparations. In addition, doses of 1/200, 1/100, 1/50, 1/20, 1/10, and 2 LD<sub>50</sub> of the Flexner extract were injected in tumor-bearing mice, and changes in rectal temperature recorded (Fig. 1).

Changes induced by exposing tumor-bearing mice and 15-day-pregnant mice for a sustained period to external heat and to cold were also investigated. An incubator maintained at 37°C constituted the heat chamber, while the cold chamber was a refrigerator kept at 4-6°C. Rectal temperature for mice kept in the incubator for 4 hours ranged between 39° and 41° C, and for mice kept in the refrigerator for 12 hours the rectal temperature range was 34-36° C.

The 200 Rockland mice used in this study were of a standardized inbred strain tested at regular intervals for freedom from intestinal infections.

**Results and Discussion.** Within a short time after the injection of  $\frac{1}{4}$  LD<sub>50</sub> of the endotoxins, a marked hypothermia developed, the rectal temperature often falling below 30° C (Fig. 1). Closely associated in time with the abrupt drop in temperature was the appearance of tumor-hemorrhage in tumor-bearing mice, and vaginal bleeding and often abortion in pregnant mice. There was no observed difference in the action of the endotoxin-containing preparations of *Shigella*, *Salmonella* or *Rhodospirillum*. Neither of the hemorrhage effects was evident in the control mice exposed to low or to high external temperatures.

Following the administration of 2 LD<sub>50</sub>, an abrupt hypothermia developed often reaching a minimum of 24° C, followed by death within 5 to 24 hours from the time of injection. Doses below 1/10 LD<sub>50</sub> did not appear to induce significant changes in rectal temperature (Fig. 1). Severe diarrhea was a uniform consequence of the injection of the 1/10,  $\frac{1}{4}$  and 2 LD<sub>50</sub> doses.

<sup>1</sup> Zahl, Paul A., and Bjerknes, Clara, *Proc. Soc. Exp. Biol. and Med.*, 1944, **56**, 153.

<sup>2</sup> Zahl, Paul A., and Bjerknes, Clara, *Proc. Soc. Exp. Biol. and Med.*, 1943, **54**, 329.

\* It is generally agreed that the toxic effects of extracts of *Shigella paradysenteriae* (Flexner) and of *Salmonella typhimurium* are referable solely to the endotoxin whose pattern of pathogenesis is characteristic of the endotoxins of most gram-negative bacteria. In view of this, we have for convenience chosen to designate our relatively unpurified preparations as endotoxins.

<sup>3</sup> Zahl, Paul A., Hutner, S. H., and Cooper, F. S., *Proc. Soc. Exp. Biol. and Med.*, 1943, **54**, 48; 1943, **54**, 187; Zahl, Paul A., and Hutner, S. H., *Am. J. Hyg.*, 1944, **39**, 189.

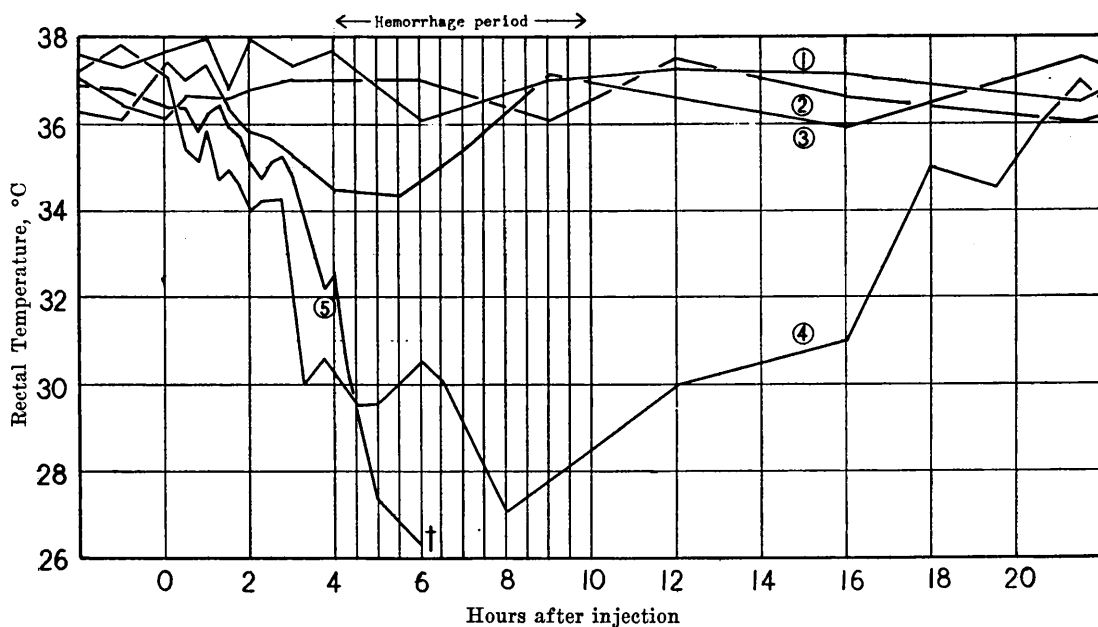


FIG. 1.

Typical temperature curves for tumor-bearing mice receiving various intraperitoneally injected doses of the endotoxin of *Shigella paradysenteriae* (Flexner). (1)—1/100 LD<sub>50</sub>; (2)—1/50 LD<sub>50</sub>; (3)—1/10 LD<sub>50</sub>; (4)—1/4 LD<sub>50</sub>; (5)—2 LD<sub>50</sub>. † = death.

There appears to be no direct causal relationship between the endotoxin-induced hypothermia in mice and the parallel histopathological changes. This view is supported by a body of data to the effect that (1) decidua-placental hemorrhage in rabbits is paralleled by hyperthermia, whereas in mice it is paralleled by hypothermia, (2) doses between 1/10 and 1/200 LD<sub>50</sub> in mice regularly induce tumor-hemorrhage,<sup>3</sup> but without appreciable concurrent thermal changes, (3) the hypothermia induced by refrigeration induces neither tumor- nor decidua-placental hemorrhage.

A number of workers<sup>4-6</sup> have reported marked hypothermia in guinea pigs, following injection of killed suspensions of various gram-negative bacteria. Feldman and Gellhorn<sup>7</sup> found that in rats a stimulation of the vago-insulin system (a portion of the parasympathetic) is followed by decrease in body

temperature and blood sugar, and that stimulation of the sympathetico-adrenal system leads to rise in both body temperature and blood sugar. From the results of injecting typhoid-paratyphoid vaccine into normal, into vagotomized, and into adreno-demedullated rats, they concluded that both systems were simultaneously stimulated, with stimulation of the sympathetico-adrenal system predominating.

Applying this explanation, it would appear that in the mouse and the guinea pig the endotoxin of gram-negative bacteria stimulates predominantly the vago-insulin system, while in the rat,<sup>7</sup> the rabbit,<sup>1</sup> the cat,<sup>8</sup> and man,<sup>8</sup> action of the sympathetico-adrenal system predominates but with evidence often of a rhythm in dominance of first one system and then the other. This hypothesis appears to be in accord with the studies of Delafield and collaborators<sup>9</sup> who observed marked initial hyper-

<sup>4</sup> Sanarelli, J., *Ann. Inst. Pasteur*, 1893, **7**, 225.

<sup>5</sup> Pfeifer, R., *Z. f. Hyg.*, 1892, **11**, 393.

<sup>6</sup> Olitsky, L., Avering, S., and Koch, P. K., *J. Immunol.*, 1942, **45**, 237.

<sup>7</sup> Feldman, J., and Gellhorn, E., *Endocrinology*, 1941, **29**, 141.

<sup>8</sup> Ranson, S. W., Jr., *Arch. Int. Med.*, 1938, **61**, 285; Ranson, S. W., Jr., Clark, C., and Magoun, H. W., *J. Lab. and Clin. Med.*, 1939, **25**, 160.

<sup>9</sup> Delafield, M. E., *J. Path. and Bact.*, 1932, **35**, 53; Cameron, G. R., Delafield, M. E., and Wilson, G., *Idem.*, 1940, **51**, 223.

glycemia followed by hypoglycemia in rabbits injected with either the crude or purified endotoxins of *Salmonella typhimurium* and many other gram-negative organisms. These changes did not occur following the injection of extracts of gram-positive organisms.

It has generally been supposed that the endotoxins of gram-negative bacteria are direct vascular poisons affecting the capillary endothelium.<sup>10</sup> However, in view of the above considerations, an alternative explanation would refer the locus of damage to the innervation of the capillaries rather than to the capillary walls directly. This point of view would link the otherwise apparently unrelated thermal and histopathological effects of the endotoxins of gram-negative bacteria.

The character of the end results, *viz.*, (1) disturbances in temperature regulation, (2) disturbances in carbohydrate metabolism, and (3) various hemorrhagic phenomena, may point also to possible damage localized at a high level of the nervous system. This level may be at the hypothalamus, in view of its recognized role in the regulation of temperature and carbohydrate metabolism. In addition, it has been repeatedly observed<sup>11</sup> that lesions of the hypothalamus induce marked hemorrhagic conditions in the gastro-intestinal mucosa, a tissue notably sensitive to damage by the endotoxins of gram-negative bacteria.

The selective character of endotoxin-

induced damage to capillaries in implanted tumors, in decidua-placental tissues, and in the gastro-intestinal mucosa can be as well explained by assuming a higher sensitivity to hypothalamic or to direct stimulation of the local innervation of these tissues, as by assuming a high sensitivity of the endothelial walls to direct toxic action. The long-known localization of colloid azo-dyes in the types of lesion under discussion is not in conflict with such a thesis. The greater permeability to large colloid particles on the part of capillary walls in these tissues may rather reflect the condition of the innervation controlling the tonus of the capillary walls.

*Summary.* The endotoxin of *Shigella paradysenteriae* (Flexner) induced in mice a marked hypothermia which developed at the same time as hemorrhage in implanted tumors and in decidua-placental tissues. Similar effects were observed for the endotoxins of *Salmonella typhimurium* and *Rhodospirillum rubrum*.

Hypothermia and hyperthermia induced in tumor-bearing and pregnant mice by exposure to high and low temperature air baths were not paralleled by observable hemorrhagic effects.

It is concluded that the endotoxin-induced hypothermia in mice and the previously reported endotoxin-induced hyperthermia in rabbits bear no direct causal relationship to the histopathological effects which develop in parallel to these thermal changes. An hypothesis based on the assumption that the endotoxins of gram-negative bacteria act as possible neural poisons rather than as exclusively vascular poisons is discussed.

<sup>10</sup> For appropriate literature citations, *vide*: Zahl, Paul A., Hutner, S. H., and Cooper, F. S., *Proc. Soc. Exp. Biol. and Med.*, 1943, **54**, 137.

<sup>11</sup> Fulton, J. F., *Physiology of the Nervous System*, Oxford University Press, N.Y., 1943, p. 241.