The Effect of Endotoxins on Frog Blood and Survival Time at Several Ambient Temperatures[1] (34880)

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A study was undertaken of the effect of several bacterial endotoxins on the blood cells of *Rana pipiens*. "Normal" reactivity of mammals to endotoxins apparently results to a substantial degree from past exposure to gram-negative bacteria (1–3), and it was hoped the study would elicit new amphibian–bacterial endotoxin interactions possibly unlike those of mammals as well as information on phylogenetic development of host–bacterial interrelationships.

Numbers, types, and percentages of blood cells in *R. pipiens* vary with season, age, and sex (4); in the present study, therefore, average cell values were first established with which data from endotoxin-treated frogs could then be compared. This report presents the effects of various bacterial endotoxins on the blood picture and survival of *R. pipiens* at several ambient temperatures.

Materials and Methods. Adult male and female *R. pipiens,* body weight 25–30 g, were used in the investigation. Total cell counts per mm^3 were made on blood taken from the ventricle of the frog using a Unopet and the hemocytometer.

Accurate counts of leukocyte numbers are impractical by the usual procedures since frog blood contains thrombocytes and nucleated erythrocytes. Therefore, differentiation of cell types was made under oil immersion on Giemsa-stained smears of blood. The latter was obtained from the lip of the frog. From 3000 to 4000 cells were counted on each smear and the percentage of thrombocytes was determined. The absolute number of thrombocytes per mm^3 was then computed by multiplying the total number of cells in 1 mm^3 by the percentage of thrombocytes. Differential leukocyte counts were obtained by dividing the number of each type of leukocyte by the total number of leukocytes counted.

LD_{50}'s were attempted on *Eschericia coli, Salmonella typhosa,* and *Brucella abortus* endotoxins using suspensions prepared in pyrogen-free saline.

Three experimental and one control animal per group were used for each assay. The endotoxin, ranging from 1 to 30 mg, was given intraperitoneally. Lethal and sublethal response to endotoxin was noted at environmental temperatures of 5, 20, 30, and 37°. Assays were repeated so that a total of 10 or 12 frogs was used per dose and temperature with each endotoxin.

The animals, weighed and examined immediately after death, showed no significant body weight and gross visceral changes, hemorrhagic necrosis at injection site, or difference in type of death at various temperatures.

The effect of intraperitoneal injection of *E. coli, S. typhosa* and *B. abortus* endotoxins on frog blood cells was studied at temperatures of 20 and 30°. An experimental animal in each group (4/group) received 1, 2, or 4 mg of endotoxin; the control was given saline. Blood smears and cell counts were made 1 hr after injection. Endotoxin effects on frog blood cells at 5 and 37° were not determined.

Results. LD_{50}'s could not be established in the frog with the endotoxins. Single injections of from 1 to 30 mg were ineffective at 5 and at 20°, and injections repeated at 2-week intervals were also without visible effect at these temperatures.

At 30° neither endotoxin-injected animals nor pyrogen-free saline controls survived, at best, beyond 4 days (Table I).

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TABLE I. Survival of Frogs at 30° Following Single Injections of *E. coli*, *S. typhosa*, and *B. abortus* Endotoxins.[a]

After treatment (days)	Untreated controls	Dose (mg)		
		1	2	4
0	10/10[b]	30/30	30/30	30/30
1	9/10	15/30	17/30	12/30
2	7/10	6/30	3/30	3/30
3	4/10	0/30	0/30	0/30

[a] Ten frogs treated with each of 3 endotoxins.
[b] Survivors/total.

At 37° no animal survived beyond 8 hr. The endotoxin-treated frogs seemed less able to withstand the elevated temperature and died within 4 hr (Table II).

Total cell counts (mainly erythrocytes) of 400,000 to 700,000/mm^3 obtained in the investigation in untreated animals are similar to red cell values reported by several investigators (4–7).

The blood picture in untreated *R. pipiens*, including blast and juvenile forms, observed in the present study, is similar to that described for this species by Jordan (8).

Percentages of types of leukocytes in the present study (Table III) generally agreed with values noted by others (9, 10) for this species.

At 20°, a twofold decrease in thrombocytes was observed in endotoxin-treated frogs (Table IV). Thrombocytes are thought to participate in blood clotting in amphibia (4, 11) and the finding may indicate endotoxin activation of amphibian clotting mechanisms similar to that reported in mammals (12) and in the invertebrate Limulus (13).

TABLE II. Survival of Frogs at 37° Following Single Injections of *E. coli*, *S. typhosa*, and *B. abortus* Endotoxins.[a]

After treatment (hr)	Untreated controls	Dose (mg)		
		1	2	4
0	12/12[b]	36/36	36/36	36/36
0.5	12/12	31/36	28/36	30/36
1	10/12	26/36	24/36	20/36
2	6/12	10/36	8/36	5/36
4	4/12	0/36	0/36	0/36

[a] Twelve frogs treated with each of 3 endotoxins.
[b] Survivors/total.

No significant change in total blood cell counts was noted either in animals given endotoxin or in untreated frogs kept at elevated temperatures.

The percentages of leukocyte types in control and experimentally-treated animals were generally similar. Eosinophils increased somewhat after endotoxin administration, but the rise was about the same for every dose used.

Discussion. Endotoxin sensitization of the frog to heat is indicated by the shortened survival time of endotoxin-treated as compared with control animals at 30 and 37°, but further study is needed to substantiate this point. Sensitization of mammals to both heat and cold by *E. coli* and *Serratia marsescens* endotoxins has been reported by Berry (14).

TABLE III. Percentage of Types of Leukocytes in the Blood of the Frog.

Cell type	Percentage
Neutrophil	35–60
Eosinophil	10–20
Lymphocyte	25–40
Monocyte	5–10
Basophil	1–5

The insusceptibility of frogs at 5° to endotoxin may be due to hibernation that requires only low metabolic acitvity. The endotoxin may affect metabolic activity in the amphibian. Bacterial endotoxins have been shown to poison liver tryptophan-pyrrolase enzyme in mammals (15, 16), and an investigation of the effect of endotoxins on frog liver metabolism might prove fruitful.

Insensitivity of *R. pipiens* to a single endotoxin injection may be due to absence of the endotoxin from the animal's environment. However, since an acquired delayed hypersensitivity, readily produced in mammals, did not result following repeated endotoxin administration, the possibility of a cellular immunity to these endotoxins in *R. pipiens* cannot be overlooked.

TABLE IV. The Effect of Single Injections of *E. coli, S. typhosa,* and *B. abortus* Endotoxins on the Numbers of Thrombocytes in the Blood of Frogs Kept at 20°.

Treatment[a]	No. of thrombocytes/mm³[b]
Untreated controls	7317 ± 129.6
Endotoxin, 1	4643 ± 88.4[c]
2	4276 ± 51.4[cd]
4	4379 ± 56.8[cd]

[a] Treatments with individual endotoxins are combined under dosage.

[b] Mean ± SE for 28 frogs in control and in each dosage group.

[c] Significantly different from controls at $p < 0.001$.

[d] 2- and 4-mg groups, combined, significantly different from 1-mg group at $p < 0.005$.

It would be of interest to determine if hypersensitivity to endotoxins normally indigenous to this species could be evoked in a manner similar to that produced with mammalian sensitive endotoxins.

Summary. Treatment of frogs with any of three endotoxins resulted in more rapid deaths at 30 and at 37° and a decrease in blood thrombocytes. The frogs were unaffected by bacterial endotoxin at 5 and at 20° at doses of 1 through 30 mg, and repeated injections at a 2-week interval were equally without effect. Other than thrombocytes, the formed elements of the blood were unchanged after endotoxin treatment.

1. Atkins, E., Physiol. Rev. **40**, 580 (1960).

2. Zweifach, B. W., and Janoff, A., Annu. Rev. Med. **16**, 201 (1965).

3. Weil, M. H., *in* "Bacterial Endotoxins" (M. Landy and W. Braun, eds.), p. 187. Rutgers Univ. Press, New Brunswick, N. J. (1964).

4. Foxon, G. E. H., *in* "Physiology of the Amphibia" (J. Moore, ed.), p. 172. Academic Press, New York (1964).

5. Kaplan, H. M., Trans. Ill. State Acad. Sci. **45**, 170 (1952).

6. Hunter, A. A., and Hunter, F. R., J. Cell. Physiol. **49**, 479 (1957).

7. Tischendorf, E., and Scheon, S., Klin. Pathol. Blut. **50**, 125 (1950).

8. Jordan, H. E., *in* "Handbook of Hematology" (H. Downey, ed.), Vol. 2, p. 760. Harper, New York (1938).

9. Goodale, P., J. Pathol. Bacteriol. **14**, 195 (1910).

10. Scarborough, R. A., Yale J. Biol. Med. **4**, 325 (1931).

11. Neumann, E., Arch. Microsk. Anat. Entwicklungsmech. **76**, 725 (1910).

12. Des Prez, R. M., Horowitz, H. I., and Hook, E. W., J. Exp. Med. **120**, 305 (1964).

13. Levin, J., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **26**, 1707 (1967).

14. Berry, L. J., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **25**, 1264 (1966).

15. Berry, L. J., and Smythe, D. S., J. Exp. Med. **118**, 587 (1963).

16. Feigelson, P., and Greengard, O., J. Biol. Chem. **236**, 153 (1961).

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