asphyxia-resistant contraction by dinitrophenol is favoured by keeping the *p*H of the saline at 6·5 (phosphate buffer). Thus formation of adenosine triphosphate is necessary not only for relaxation, but also for keeping the muscle relaxed.

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Toxicity of Endotoxin and Snake Venom in the Hagfish

REACTIONS to parenteral administration of endotoxin in mammals include pyrexia, leukopænia followed by leukocytosis, vasodilation, and irreversible shock[1]. Spink[2] reviewed the actions of snake venom and endotoxin, and concluded that their mechanism of action is similar; however, in recent studies Condie *et al.*[3] showed that there are few parallels between the biological responses of rabbits to *Escherichia coli* endotoxin and *Agkistrodon piscivorus* venom and that the two toxins are distinct immunochemically.

Stetson[4] postulated that the reactions to endotoxin may reflect hypersensitivity in animals the enteric flora of which include Gram-negative organisms. Since earlier studies[5] in the California hagfish, *Eptatretus stoutii*, indicated that this primitive vertebrate is essentially anergic and unable to develop either immediate or delayed hypersensitivity to bacterial or virus antigens, we felt that a comparison of the toxic action of snake venom and endotoxin in this species would be of interest.

Freshly obtained California hagfish, weighing 30–80 gm., were kept in well-aerated sea-water, maintained at 10° C., for a preliminary 24-hr. observation period, and then divided into groups of two animals per dose of toxin. The doses of *Agkistrodon piscivorus* (water moccasin) and *Naja flava* (yellow cobra) venoms and *Escherichia coli* endotoxin were made up to 2 ml. volumes in 0·9 per cent saline, and injected into the subdermal lateral sinuses in the tail region of the fish, anterior to the caudal heart. This approximated an intravenous injection in this animal, which has an intermediate-type open circulation, with return vessels similar to *Amphioxus*. Ten-fold variations in dosage were studied (Table 1), and dramatic differences in response to endotoxin and snake venoms were noted.

Endotoxin, even in the 100 mgm. dose, failed to produce a detectable reaction in the hagfish, and the animals remained coiled up, their normal attitude when undisturbed. There were no observable differences between these endotoxin-treated fish and the control animals receiving 2 ml. of 0·9 per cent saline. By contrast, all doses of the venoms, except 1 mgm. of *Agkistrodon piscivorus* venom, were lethal within 5 hr., and the highest doses produced death within 30 min. The dying animals uncoiled to a straightened position, and swimming movements stopped. The complete suppression of these movements suggests action of the venom on the nervous system, since Campbell[6] has shown that swimming movements of segments continue after the hagfish spinal cord is sectioned. The isolated aneural heart of the hagfish has been studied by one of us (D. J.)[7], and the effects of snake venom on this organ are now being tested and will be reported later.

Table 1. RESPONSE TO ENDOTOXIN AND SNAKE VENOMS IN THE HAGFISH

Toxin dose	Deaths* 1 hr.	24 hr.	48 hr.
Agkistrodon piscivorus venom (mgm.)			
1	0/2	0/2	0/2
10	0/2	2/2	—
100	2/2	—	—
Naja flava venom (γ)			
50	2/2	—	—
100	2/2	—	—
1,000	2/2	—	—
Escherichia coli endotoxin (mgm.)			
1	0/2	0/2	0/2
10	0/2	0/2	0/2
100	0/2	0/2	0/2

* No. of deaths within the period specified after administration of the toxin.

Table 2. RESPONSE TO ENDOTOXIN AND SNAKE VENOMS IN THE RABBIT (1 KGM.)

Toxin dose	Deaths* 1 hr.	24 hr.	48 hr.
Agkistrodon piscivorus venom (mgm.)			
1	1/5	1/5	1/5
10	9/10	9/10	9/10
Naja flava venom (γ)			
100	0/10	0/10	0/10
200	0/4	4/4	—
500	0/4	4/4	—
Escherichia coli endotoxin (mgm.)			
1	1/20	1/20	1/20
10	2/5	3/5	4/5
100	1/3	1/3	2/3

* No. of deaths within the period specified after administration of the toxin.

That a 100-mgm. dose of endotoxin was without effect in these animals is of interest when compared to the effect in rabbits (Table 2). The dose in the hagfish is equivalent to about 1·5 gm./kgm., and the consistent lethal effect of 1/10, and often of 1/100, of that amount in rabbits is important in dissociating the activities of endotoxin and snake venom.

The hagfish may be immune to endotoxin because of unknown natural or tissue inhibitors. Alternatively, they may be able to break endotoxins down to less toxic materials. However, in view of the extremely high doses of endotoxin used in this study, we believe that these hypotheses are less attractive than the Stetson theory that reactions to endotoxin in mammals are a result of hypersensitivity to the products of gut tract flora. This hypothesis could be tested further in germ-free mammals using toxins and dosages comparable with those used in this work.

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