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Rôle of Bacteria in the Production of Tourniquet Shock in Rats.*

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Recently, Aub and coworkers[1] suggested that infection with *Cl. welchii* might be the cause of tourniquet shock. Ligation in dogs of the triceps surae muscles and then release after 5 hours, yielded toxic edema fluid contaminated with anaerobic organisms of the gas gangrene group. Intravenous injection of such fluid into normal dogs, in amount equivalent to that yielded by the donor dog, resulted in fatal shock in 9 out of 32 animals.

Prinzmetal *et al.*[2] produced shock in dogs by removal, crushing, and replacement of the quadriceps muscle. This was attributed to absorption of bacterial toxins from the crushed muscle. Shock was prevented by the local or systemic use of sulfamerazine.[3]

Method I. High unilateral rubber band tourniquets were used to interrupt the blood supply to one hind limb of 38 adult white male rats for 5 hours. Fatal shock developed after removal of constriction. The time of survival after tourniquet release ranged from 2 to 8 hours and averaged 3½ hours.

Immediately after death the injured limb and adjacent region were shaved, immersed about 5 minutes in 70% alcohol, and covered with tincture of iodine. Samples of muscle, each several mm in diameter, were then removed aseptically from the limb, placed in culture media and incubated at 37°C as follows: (a) anaerobically in Brewer's thioglycollate medium, (b) anaerobically in meat-infusion broth enriched with 0.5% dextrose, incubated in a Brewer anaerobic jar[4] utilizing an atmosphere of illuminating gas,[5] (c) aerobically in meat-infusion broth. The heart's blood was cultured in similar manner in all rats.

The tubes remained in the anaerobic jar for 4 days before removal. Bacterial stains were made after 4 days, 8 days, and before discarding at the end of 10 days.

II. Tourniquet shock was produced in rats pretreated with: (a) *sodium sulfadiazine,* 150 to 200 mg in 0.5 cc distilled water, intraperitoneally 30 minutes before removal of tourniquet; (b) *sodium salt of penicillin,* 1000 to 1500 units intramuscularly 6 hours before tourniquet release, and every 3 hours thereafter in one group of rats and every 2 hours in a second group; (c) *polyvalent gas gangrene antitoxin,* intramuscularly 6 hours before removal of tourniquet. Five or 10 times the amount of antitoxin proportional to the human dosage was given.

Fifteen rats were treated with sulfadiazine, 18 with penicillin, and 18 with antitoxin. Twenty untreated rats served as controls.

Results. I. Anaerobic cultures of muscle from the hind limb were negative in 29 of the 38 rats. Aerobic culture of muscle was negative in 34 animals. Anaerobic and aerobic cultures of the heart's blood were negative in 29 and 32 rats respectively. The positive anaerobic and aerobic cultures from both muscle and blood consisted of colon bacilli, non-hemolytic staphylococci, non-hemolytic streptococci, or diphtheroids. Most of these organisms appeared after 4 days of culture and were probably contaminants. *Cl. welchii* was not obtained in any instance.

In 10 control rats which received 1 cc of a 24-hour culture of *Cl. welchii* intramuscularly

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1 Aub, J. C., Brues, A. M., Dubos, R., Kety, S. S., Nathanson, I. T., Pope, A., and Zamecnik, P. C., *War Med.*, 1944, **5**, 71.

2 Prinzmetal, M., Freed, S. C., and Kruger, H. E., *War Med.*, 1944, **5**, 74.

3 Freed, S. C., Kruger, H. E., and Prinzmetal, M., *Surgery*, 1944, **16**, 914.

4 Brewer, J. H., *J. Lab. and Clin. Med.*, 1939, **24**, 1190.

5 Brewer, J. H., and Brown, J. H., *J. Lab. and Clin. Med.*, 1938, **23**, 870.

TABLE I.
Results of Treatment.

Treatment	No. of rats	No. dead in shock	Mortality (%)	Time of survival (hr) range	mean
Sulfadiazine	15	15	100	2-8	4
Penicillin	18	17	94	1-9	3¾
Gas gangrene antitoxin	18	17	94	2-9	4
None (controls)	20	19	95	2-13	4¾

in the hind limb just prior to tourniquet release, the organism was readily recovered after death by anaerobic culture of muscle.

II. Treatment with sulfadiazine, penicillin, or gas gangrene antitoxin had no appreciable effect on mortality or time of survival (Table I). The blood sulfadiazine levels of 7 rats at death ranged from 4 to 56.8 mg per 100 cc.

Discussion. The experiments of Aub and associates[1] involved open operative procedures in which bacterial contamination was possible, although organisms already present in the muscle may have been the source of infection. Shock was not produced in their donor animals but rather a state bordering on it. In the studies of Prinzmetal *et al.*[2] shock did not develop until 24 hours after replacement of muscle and death occurred after 2 or 3 days. This slowly developing form of shock due to bacteria is distinct from the acute form produced by tourniquet. In the latter, shock and death occur so rapidly as to make it unlikely that bacterial toxins are contributory.

Conclusion. In the experiments described in this article infection with *Cl. welchii* or other organisms did not prove to be a significant factor in the production of tourniquet shock in rats since:

(1) Anaerobic and aerobic cultures of muscle from the hind limb after death were usually sterile.

(2) Pre-treatment with sulfadiazine, penicillin, or gas gangrene antitoxin did not influence the development of shock, mortality or time of survival.

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Three New Salmonella Types: *S. richmond, S. daytona* and *S. tallahassee.**

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A. *S. richmond.* The sole representative of this type was isolated from the feces of a child affected with mild gastroenteritis. The isolation and preliminary examination of the culture was carried out by Mr. Forrest Spindle in the laboratories of the Virginia State Department of Health. Mr. Spindle later examined stool specimens of the remaining members of the child's family and investigated possible sources of infection. No carriers were found and the source of the infection was not determined.

The organism possessed the usual cultural and biochemical attributes of the Salmonella group. Glucose, arabinose, xylose, rhamnose, trehalose, mannitol, dulcitol, sorbitol and inositol were fermented with the production of acid and gas within 24 hours, while cel-

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