4. Braun, W., Nakano, M., Freedman, H. H., Fed. Proc., 1966, v25, 370.

5. Freedman, H. H., Braun, W., Proc. Soc. Exp. Biol. & Med., 1965, v120, 222.

6. Bacterial Endotoxins, Landy, M., Braun, W., ed., Rutgers Univ. Press, 1964.

7. Jerne, N. K., Nordin, A. A., Henry, C., in Cell-bound Antibodies, Wistar Inst. Press, 1963, p109.

8. Ribi, E., Anacker, R. L., Fukushi, K., Haskins, W. T., Landy, M., Milner, K. C., in Bacterial Endotoxins, Landy, M., Braun, W., ed., Rutgers Univ. Press, 1964, p16.

9. Lee, L., Stetson, C. A., Jr., J. Exp. Med., 1960, v111, 761.

10. Schaedler, R. W., Dubos, R. J., ibid., 1961, v113, 559.

11. Braude, A. I., Siemienski, J., Bull. N. Y. Acad. Med., 1961, v37, 448.

12. Stetson, C. A., Jr., Bact. Rev., 1961, v25, 457.

Received February 23, 1967. P.S.E.B.M., 1967, v125.

Effect of Evisceration on the Development of Tourniquet Shock.* (32153)

JOHN KABAL AND ESTELLE R. RAMEY (Introduced by L. S. Lilienfield)
Department of Physiology and Biophysics, Georgetown University, Schools of Medicine & Dentistry, Washington, D. C.

The whole concept of the role of 'toxic' factors in the development of the shock picture has been widely argued. For example, the lethality of bilateral limb ischemia is said to be caused by toxic factors arising from the injured limbs. This view is based on the observation that survival time of tourniquet stressed animals is extended following the release of the tourniquet if the tourniquet is reapplied within a critical period(1). This could be interpreted to mean either that the toxic factor theory is untenable or that a critical quantitative relationship exists between the amount of toxic factor released and the precipitation of the shock state. None of these so-called toxic factors have been identified from traumatized limbs and the question remains unresolved. The most widely held theory of the mechanism leading to the development of shock relates to the pooling of fluid in the traumatized area. There is an extensive literature to support this concept(2). We have data (to be reported later) which tends to support the hypothesis that massive extravasation of fluid from limb blood vessels damaged by prolonged anoxia, leads to a diminution in circulating blood volume with subsequent hypotension and vascular collapse.

Another line of evidence suggests that the mesenteric hyperemia and local intestinal hemorrhages observed following traumatic shock result in anoxic damage to gut cells leading to increased permeability of toxic substances(3). If this were the case, it might be postulated that removal of the source of such factors, *i.e.*, evisceration, could alter the survival time of tourniquet stressed animals and extend this to the survival time of the eviscerated animal without tourniquet. This study is an attempt to verify this hypothesis. We eviscerated tourniquet stressed animals and compared their survival time to non-eviscerated animals with hind limb ischemia. We included adrenalectomized animals in this study because of their well known stress sensitivity. It was felt that if the gut toxins were contributing significantly to the development of tourniquet shock, this might be markedly demonstrated in an animal with a very small range of homeostatic responses.

Methods. Male, albino rats of Sprague-Dawley strain, 225 ± 25 g, were used throughout the experiment. The animals were about 10 weeks old.

The animals were maintained on Purina Rat Chow and drinking water *ad libitum*. The room temperature was maintained at 72 ± 2°F. The rats were kept in individual cages.

Limb ischemia was produced by ligation of the hind limbs at the inguinal level with a double rubber band under light ether anesthesia. The ligature was kept in place for 5 hours.

* Supported in part by U.S.P.H.S. Grant AM-04714.

TABLE I.

Normal groups	No. of rats	Survival time in hr, mean ± S.D.	Significance p value
1. Bilateral limb ischemia	21	11.24 ± 1.00	—
2. Evisceration	18	27.27 ± 4.71	to 1: <.01
3. Evisceration & simultaneous tourniquet	14	9.61 ± 1.54	to 2: <.01
4. Evisceration after tourniquet removal	16	10.35 ± 1.94	to 2: <.01

TABLE II.

Adrenalectomized groups	No. of rats	Survival time in hr, mean ± S.D.	Significance p value
1. Bilateral limb ischemia	26	1.92 ± .56	—
2. Evisceration	18	9.15 ± 1.72	to 1: <.01
3. Evisceration & bilateral limb ischemia	23	1.55 ± .39	to 2: <.01

Evisceration was performed under ether anesthesia. After laparotomy, the intestine was separated from the mesenterium, ligated and excised between the pylorus and rectum. No significant bleeding was observed. The wound was securely sutured. At the end of the experiment the abdominal cavity was checked for bleeding. Evisceration was performed at two different times: (a) at the time the tourniquets were applied, and (b) when the tourniquets were removed.

Adrenalectomy was produced by the method of Ingle and Griffith(4) using the dorsal approach. The wound was closed by metal clips. No steroids were administered. The animals were used after the seventh postoperative day. They were maintained on Purina Rat Chow and 0.9% NaCl in drinking water *ad libitum*. Adrenalectomy was confirmed at autopsy.

We calculated the standard deviation and the significance of the difference between two means using the "Student T Test."

Results. Table I shows the results of the first series of experiments. After 5 hours of bilateral limb ischemia, all animals died approximately 11 hours after removal of the tourniquet. Evisceration was combined with 5-hour limb ischemia either when the tourniquets were applied or after they were removed. No significant differences were observed in the survival times compared to the intact, tourniquet stressed group.

In the next series of experiments, we used adrenalectomized animals. As expected, their survival time was approximately 1/5 of the normal tourniquet shocked rats (Table II). Survival time after evisceration was about 1/3 of the non-adrenalectomized, eviscerated group. When limb ischemia was combined with evisceration (performed when the tourniquets were taken off) in adrenalectomized rats, the survival time did not differ significantly from the adrenalectomized, tourniquet stressed rats.

Discussion. There is considerable evidence to show that during shock the intestinal mucosa is metabolically altered and becomes more permeable to many substances including possible toxic agents(5). It has been suggested, therefore, that the damaged intestines play a causative role in the deterioration and death of the severely stressed animal(6). It had been observed(2) that dogs subjected to hemorrhagic shock exhibited a marked depression of oxygen uptake by the gut even when blood was reinfused and the mesenteric blood flow returned to normal. Oxygen consumption in other tisssues (heart, limbs, liver) returned to normal under these conditions but the metabolism of the gut did not and these animals were later found to be in irreversible shock. The intestinal mucosa of these animals was found to be defective in terms of ATP synthesis and oxidative phosphorylation with a concomitant increase in permeability to many substances, including toxins. This suggested the possibility that the damaged gut might be the site of release into the blood of non-specific toxic materials whenever a sufficiently severe stress was imposed on the animal. These toxins could then play

an important part in the onset of the shock state. We used tourniquet induced limb ischemia as a stress and were able to obtain consistent data on the survival time of such animals when the tourniquet had been in place for 5 hours and then removed. The intestines of such animals, dying in shock within 11 hours after removal of the tourniquet, reveal marked hyperemia of the mesenteric veins. Evisceration, either before or after removal of the tourniquet seemed to have no effect on survival time of such animals. Evisceration itself is a fatal procedure but animals subjected only to evisceration have more than twice the survival time of animals subjected to tourniquet application alone. It might be expected that the combined traumatic procedures would significantly shorten the survival time observed with either stress. This did not occur. Animals subjected to both stresses lived as long as those animals subjected to tourniquet alone. The response of the adrenalectomized animal was of interest because of the stress sensitivity of this preparation. Despite the fact that the adrenalectomized rats had a greatly diminished survival time when subjected to tourniquet stress, the additional trauma of evisceration did not further embarrass survival. Evisceration alone in these animals had a less acute effect on survival than did the production of bilateral limb ischemia. In no case was there any indication that the presence of toxic factors from the gut played a major role in the demise of the animal.

These data do not, therefore, support the hypothesis that one of the factors in the onset of shock and death in tourniquet stressed animals is the release of toxic factors from a concomitantly injured gut.

Summary. Limb ischemia produced by bilateral hind limb tourniquets in rats results in 100% mortality in intact animals within 11 hours after removal of the tourniquet. It has been suggested that one factor contributing to the demise of the animals is the production of toxic factors from the hyperemic gut. We tested this hypothesis by eviscerating normal and adrenalectomized rats either before or after the application of the tourniquet. Evisceration itself was tolerated for a 24-hour period. Evisceration, however, had no significant effect on survival time of tourniquet stressed animals. It is, therefore, unlikely that factors produced by the gut in the intact or adrenalectomized animal contributes significantly to the life threatening effects of tourniquet shock.

1. Meyer, R. K., McShaw, W. H., Goldman, A., Shipley, E. G., Am. J. Physiol., 1946, v147, 66.

2. Stoner, H. B., in Recent Progress and Present Problems in the Field of Shock. Fed. Proc., 1961, suppl. No. 9, 43.

3. Gurd, F. N., Am. J. Surg., 1965, v110, 333.

4. Ingle, D. J., Griffith, J. Q., The Rat in Laboratory Investigation, Lippincott, Philadelphia, Pa., 1942, Chap. 16.

5. Nelson, R. M., Noyes, H. E., Surgical Forum (38th Congress), 1953, 474.

6. Sweinburg, F. B., Fine, J., J. Exp. Med., 1960, v112, 793.

Received February 24, 1967. P.S.E.B.M., 1967, v125.