propionate in the damaged patient are discussed.

A part of this report is based on work done by Capt. Braasch in the Department of Physiology of the University of Illinois School of Medicine in partial fulfillment of the requirements for the degree of Master of Science in Physiology. The authors wish to thank the Schering Corporation for a generous supply of testosterone propionate.

Received July 21, 1950. P.S.E.B.M., 1950, v75.

## Blood Amino Acid Level and Adrenal Cholesterol Content During "Tourniquet Shock" in the Rat. (18140)

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It has been established that "shock" will produce an elevation of the blood amino acid level and a decrease in adrenal cholesterol content(1,2). The "sparing" effect of administration of isotonic sodium chloride solution in lowering mortality in traumatic shock has been recorded by various investigators (3-5). It was of interest to determine the effects of saline therapy on the blood amino acid level and the adrenal cholesterol content of rats in "tourniquet shock." The findings of this investigation are presented in this paper.

*Experimental.* White male albino rats of the Sprague-Dawley strain, weighing 220 to 290 g and fed on Purina dog chow checkers, were used. The procedure for shock production in mice developed by Rosenthal(4) and used by Harkins(5) for rats, was employed (6). The animals were fasted for 16 hours before the experiment but were permitted water. With the animals under light ether anesthesia, elastic rubber band (Eberhard Faber No. 30) tourniquets were applied to the limbs and the teeth were clipped to prevent tourniquet removal or self-laceration. The animals were placed in individual cages without access to food or water. Depending on the type of experiment, the animals were sacrificed by decapitation without tourniquet release or tourniquets were removed and the animals replaced in the individual cages to be sacrificed at varying periods of time following release. For the experiments reported here, the occlusion was of both hind-limbs for 4½ hours and of hind- and fore-limb unilaterally for 3 hours or for 5 hours. For treatment of the induced "shock," a 0.9% NaCl solution was given intraperitoneally, 10 ml immediately following tourniquet release, 10 ml 2 hours later and 3½ hours after release the amount necessary to bring the total administered to 10% of the body weight. All experiments were conducted at room temperatures of 20 to 25°C. After decapitation of the animal, the adrenal glands were immediately dissected, weighed and analyzed for cholesterol according to the method of Schoenheimer and Sperry(7). The total cholesterol was determined in each case and the amount of cholesterol is reported as mg/100 mg of fresh wet adrenal tissue. Blood amino acid(8), urea(9), and hematocrit determinations were carried out on blood ob-

1. Davis, H. A., Shock and Allied Forms of Failure of the Circulation. New York, Grune and Stratton, 1949.

2. Wilhelmi, A. E., *Ann. Rev. Physiol.*, 1948, v10, 259.

3. Meyer, J., Lendrum, B., and Katz, L. N., *Am. J. Physiol.*, 1945-46, v145, 151.

4. Rosenthal, S. M., *Pub. Health Rep.*, 1943, v58, 1429.

5. Harkins, H. N., *Am. J. Physiol.*, 1947, v148, 538.

6. Hamolsky, M. W., Gierlach, Z. S., and Jensen, H., *Am. J. Physiol.*, in press.

7. Schoenheimer, R., and Sperry, W. M., *J. Biol. Chem.*, 1934, v106, 745.

8. Frame, E. G., Russell, J. A., and Wilhelmi, A. E., *J. Biol. Chem.*, 1943, v149, 255.

TABLE I. Blood Amino Acid and Urea Levels and Hematocrits at Different Time Intervals After Release of Tourniquets Applied on Both Hind Legs for 4.5 Hr. Average values ± standard deviation for No. of animals indicated in parentheses.

| Time after release | Amino acid nitrogen (mg %) | Urea nitrogen (mg %) | Hematocrit, % |
|---|---|---|---|
| (Normal values) | 12.3 ± 0.66 (14) | 15.0* | 45.7 (5) |
| 0 | 10.8 ± 2.59 (18) | 14.6 ± 2.91 (12) | 45.8 (17) |
| 30 min. | — | — | 59.9 (11) |
| 1 hr | 13.75 ± 1.38 (13) | 20.8 ± 3.89 (10) | 61.1 (8) |
| 2 " | 16.15 ± 2.15 (11) | 27.9 ± 2.65 (3) | 61.3 (3) |
| 4 " | 18.0 ± 0.77 (4) | 39.1 ± 0.22 (2) | — |
| 9 " | 20.0 ± 2.03 (11) | 55.0 ± 11.51 (3) | — |
| 16–17 " | 20.7 ± 1.71 (12) | 98.4 ± 41.70 (7) | 67.0 (5) |

* Taken from Fraenkel-Conrat, J., Fraenkel-Conrat, H., and Evans, H. M., *Am. J. Physiol.*, 1942, v137, 200.

tained at the time the animals were killed by decapitation.

*Results and discussion.* By the method described, it was found that occlusion of both hind-limbs for 4½ hours or hind- and fore-limb unilaterally for 3 or 5 hours produced a shock-like state in all animals after the tourniquets were removed. These time periods yielded a satisfactory 24-hour mortality percentage for the purpose of this study. With tourniquets on both hind-limbs for 4½ hours, mortality 24 hours after tourniquet release for the untreated group averaged 89.5% compared with 9.76% average mortality rate for the saline treated animals(6). Occlusion of hind- and fore-limb unilaterally for 3 hours resulted in the death, in the 16 hours following tourniquet release, of 70% of the non-treated animals compared with an 18% average mortality rate in the saline treated animals. Tourniquets on hind- and fore-limb unilaterally for 5 hours produced an average mortality, 16 hours after tourniquet release, of 84% in the non-treated group and 10% in the saline treated group. The mortality figures, obtained both for treated and untreated animals, agree well with those recorded in the literature(3-6). The stress, produced by either occlusion of both hind-limbs for 4½ hours or hind- and fore-limb unilaterally for 5 hours, seems to be approximately of the same magnitude.

The data presented in Table I show that after tourniquet release at different time intervals, an early and progressive elevation in the blood amino acid and urea nitrogen concentration occurs. The values for blood amino acid and urea nitrogen, obtained at the time of tourniquet release, (zero time), agree with those for normal untreated animals. From these results and those of experiments 4 and 8 (Table II), it is evident that the application of tourniquets *per se* for the time periods employed does not produce any elevation in these blood constituents. Blood amino acid nitrogen determinations on 24 animals sacrificed at varying intervals up to 22 hours without tourniquet release showed no changes from the normal control values. Apparently only after the release of the tourniquets can this change in blood amino acid and urea nitrogen concentration be observed (Table I and Exp. 5, 6, 9 and 11 of Table II).

Apparently, occlusion of the limbs for a period of several hours is, itself, a sufficient stress producing procedure to cause a lowering of the adrenal cholesterol content (Exp. 4 and 8 of Table II). On release of the tourniquets, there is a further progressive reduction in the adrenal cholesterol level (Exp. 5, 6, 10 and 11 of Table II). The finding that occlusion of the limbs *per se* produces no change in the blood amino acid and urea nitrogen concentration in spite of a definite increased activity of the adrenal cortex may perhaps be explained as follows: during the period of limb occlusion the organism is still capable of managing properly the accelerated formation of protein breakdown products brought about by the increased activity of the adrenal cortex. However, release of the tourniquets leads to an impairment in the

9. Van Slyke, D. D., and Cullen, Y. E., *J. Biol. Chem.*, 1916, v24, 117.

TABLE II. Effect of Saline Administration on Blood Amino Acid Level and Adrenal Cholesterol Content During Tourniquet Shock. Average values ± standard deviation for No. of animals indicated in parentheses.

| No. of exp. | Manipulation and therapy | Blood amino acid nitrogen, mg % | Cholesterol content of fresh adrenal tissue, mg % |
|---|---|---|---|
| | A. Controls—no tourniquets. | | |
| 1 | None | 12.0 ± .40 (9) | 4.33 ± .89 (9) |
| 2 | Saline treatment | 11.2 ± .77 (6) | 4.75 ± 1.70 (9) |
| 3 | Anesthetized, teeth clipped, sacrificed 19 hr later | 11.6 ± .62 (4) | 4.75 ± .74 (4) |
| | B. Tourniquets on hind- and fore-limb unilaterally for 3 hr, anesthetized and teeth clipped. | | |
| 4 | Sacrificed at 3 hr, no t.r.* | 12.5 ± .63 (10) | 2.79 ± .46 (9) |
| 5 | Sacrificed 2 hr after t.r. | 15.1 ± 1.01 (9) | 1.61 ± .42 (10) |
| 6 | Sacrificed 16 hr after t.r. | 21.1 ± 3.70 (16) | .82 ± .32 (21) |
| 7 | Sacrificed 16 hr after t.r. Saline treatment | 13.9 ± 2.85 (17) | 1.23 ± .36 (19) |
| | C. Tourniquets on both hind-limbs for 4.5 hr anesthetized and teeth clipped. | | |
| 8 | Sacrificed at 4.5 hr, no t.r. | 10.8 ± 2.59 (18) | 2.63 ± .53 (8) |
| 9 | Sacrificed 2 hr after t.r. | 16.1 ± 2.15 (11) | |
| | D. Tourniquets on hind- and fore-limb unilaterally for 5 hr anesthetized and teeth clipped. | | |
| 10 | Sacrificed 2 hr after t.r. | — | 1.68 ± .58 (11) |
| 11 | Sacrificed 16 hr after t.r. | 17.9 ± 3.31 (8) | .73 ± .19 (13) |
| 12 | Sacrificed 16 hr after t.r. Saline treatment | 14.7 ± 3.76 (14) | .96 ± .27 (14) |

* Tourniquet release.

utilization and excretion of the protein breakdown products.

As can be seen from Table I, occlusion of the limbs *per se* does not cause any change in hematocrit (value obtained at zero time agrees with the normal value). However, release of the tourniquets produces a prompt increase in hematocrit. The elevated blood amino acid and urea levels, observed after tourniquet release, are not due to hemoconcentration alone since the hematocrit increase does not parallel that of the blood amino acid and urea levels.

Exp. 6, 7, 11 and 12 in Table II indicate that the "sparing" effect of saline administration to rats in "shock" is associated with a decrease in the elevation of blood amino acid concentration and in the fall of the adrenal cholesterol content. The nature of the underlying mechanisms of the "sparing" effect of saline administration in reducing mortality is at present obscure. It is quite possible that the administration of saline leads to a correction of the disturbances of body fluids and electroyte balance brought about by the release of the tourniquets. It is to be noted that saline therapy does not reverse completely the disturbances in blood amino acid concentration and adrenal cholesterol content by the time the animals were sacrificed.

*Summary and conclusions.* A shock-like state was produced in rats by applying rubber band tourniquets to the limbs for a certain length of time and then releasing them. A progressive increase in blood amino acid and urea nitrogen occurred after the release of the tourniquets. Application of the rubber band *per se* had no effect on these blood constituents but was found to cause a definite stimulation of the adrenal cortex as indicated by a lowering of the adrenal cholesterol content. Release of the tourniquets produced a further decline in the adrenal cholesterol content. The "sparing" effect of 0.9% NaCl administration in lowering mortality of rats during "tourniquet shock" was found to be associated with a decrease in the elevation of blood amino acid concentration and in the fall of the adrenal cholesterol content when compared with untreated animals.

Received September 11, 1950. P.S.E.B.M., 1950, v75.