must have contributed heavily to the weight of the bursa, the weights of the 8 bursæ containing paraffin do not constitute acceptable data concerning the influence of mechanical distention upon the weight of the bursa; therefore these data are not presented in Table II. Furthermore, in the remarks which follow, only data other than these are considered.

Discussion and conclusion. Regression of the bursa induced by unilateral castration (mechanical factor) was less than that induced by bilateral castration (hormonal and mechanical factors); compare Rats 14 and 15 and Rats 22, 23, and 24. In 6 of 7 rats which had been bilaterally castrated, the data suggest that regression of the bursa was hindered by the presence of such structures as the epididymis (mechanical factor). It is concluded that in the adult rat the weight of the bursa is influenced by two synergistic factors: (1) the hormonal action of testicular androgen and (2) the mechanical action of the contents of the bursa.

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Acute Anatomic Breakdown of Motor End Plates in Hemorrhagic Shock.*

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The pathology of the motor end plates in voluntary muscle during hemorrhagic shock is unknown. The effects of strong electric and chemical shock,[1] poliomyelitis,[2], pneumonia, other acute infections,[3] and lactic acid,[4] in producing either a local or general loss of structural and functional innervation at the myoneural junctions, have been demonstrated. Shock caused by hemorrhage,[5] trauma,[6] or gravity,[7] produces a state of anoxia followed by an accumulation of lactic acid (hyperlactacidemia) and keto acids (pyruvic, acetoacetic, etc.) in the blood. Accumulation of lactic acid increases the neurohumoral stimulant by retarding the destruction of physiologically deposited acetylcholine at the synapses.[8] The claim is made that there are no definite grounds for differentiating between hemorrhagic shock and shock from other causes.[9] The structural alterations of the neuromuscular apparatus during shock from any cause, however, remain an unexplored field.

Methods. Thirty albino rats weighing from 150 to 250 g were used. All experiments were performed after anesthesia with sodium pentobarbital (nembutal), 4 mg per 100 g intraperitoneally injected. Shock was induced by bleeding from the cut tails according to the method of Engel, Winton, and Long.[10] Blood was removed, over a period of one hour,

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1 Carey, E. J., *Am. J. Path.*, 1942, **18**, 237; *Ibid.*, 1944, **22**, 341.

2 Carey, E. J., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 3.

3 Carey, E. J., unpublished observations.

4 Carey, E. J., and Massopust, L. C., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 194.

5 Govier, W. M., and Greer, C. M., *J. Pharm. and Exp. Ther.*, 1941, **73**, 321.

6 Gutmann, H., Olson, W. H., Kroll, H. H., Levinson, S. O., and Necheles, H., *Am. J. Physiol.*, 1941, **133**, 309.

7 Cole, W. H., Allison, J. B., Leathem, J. H., Nastuk, W. L., and Anderson, J. A., *Anat. Rec.*, 1942, **84**, 468.

8 Gesell, R., Brassfield, C. R., and Hansen, E. T., *Fed. Proc.*, 1942, **1**, 29; Hansen, E. T., Worzniak, J. J., and Gesell, R., *Ibid.*, 1942, **1**, 36.

9 Weston, R. E., Janota, M., Levinson, S. O., and Necheles, H., *Am. J. Physiol.*, 1943, **138**, 450.

10 Engel, F. L., Winton, M. G., and Long, C. N. H., *J. Exp. Med.*, 1943, **77**, 397.

equivalent to 3 to 4% of the body weight. After shock had been established no further anesthesia was necessary. It was impossible to produce shock in all rats by the withdrawal of a standard amount of blood over a one-hour period because of individual differences inherent in each rat. The clinical criteria for shock were pallor, cyanosis, cold extremities, tachypnea, sluggish or absent blood flow from cut tail, increased salivation and lacrimation, and, after 6 to 12 hours, so-called bloody tears or "chromodacryorrhea" in those animals in irreversible shock. There was muscle weakness, hypotonia, atonia, and flaccid paralysis of certain voluntary muscles, in some cases preceded by coarse tremors. In some animals certain muscles were in a state of antemortem rigidity and failed to respond to either direct or indirect electrical or mechanical stimulation. Fourteen of the 30 animals died in profound shock within 24 hours. Five normal control animals were maintained under nembutal and 5 under ether anesthesia for the same periods as were the bled rats. The following muscles, either of the right or of the left side, were removed from each of 5 living rats at 2- and 4-hour intervals after bleeding began: sternomastoid, triceps, intercostals, rectus abdominis, rectus femoris, gastrocnemius, quadriceps femoris. These muscles were processed at once, by many methods, especially the gold technic, and later teased.[11] Over 1200 teased preparations were used in this study. The muscles from rats that survived the first 24-hour period were not used in this study. Blood pressures were determined in several instances by direct cannulation of the carotid artery, heparin being used as an anticoagulant. The pressure was read on a mercury manometer. Heparin in saline was used in the system.

Results. The normal structural variations in the motor end plates of the sternomastoid and intercostal muscles were established previously.[2-4] During the first 2 hours after bleeding began, the majority of the end plates were retracted into globoid masses surrounded by Kühne's granules; both the masses and the granules took an intense impregnation with gold. The epilemmal axon was beaded and at its junction with the hypolemmal axon there was either a fine filament or a large droplet of the axon. When the connection between epilemmal and hypolemmal axons was a fine filament, the center of the end plate was open and decreased in gold impregnation. When the end plate in full surface view had a large dark droplet at the connecting link with the epilemmal axon there was a wheel appearance with a large dark hub formed by the axonic droplet.

From 2 to 6 hours after bleeding began, the majority of the end plates were expanded and composed of fine ramifications depleted of Kühne's granules. Those animals in irreversible shock for 6 to 24 hours had a progressive granulation leading to liquefaction of the majority of the motor end plates. In different end plates of a single motor tree, the large gold staining droplets of the epilemmal axons could be traced in different locations progressing through the hypolemmal axons of the end plate and then projected out into the related muscle fibers.

Hemorrhagic shock developed conditions in which, instead of the fine granules of Kühne, large masses of axonic substance penetrated the end plate because of the increased permeability. These axonic materials in muscle formed either large amorphous masses darkly impregnated with gold and measuring from 10 to 200 microns in length and from 5 to 25 microns in width, or small oval or fusiform bodies that had a definitely cross striated structure which did or did not agree with the periodicity of the muscle fiber. These axonic masses projected into the muscle fiber and disconnected from the motor tree were comparable to what was previously described as "inclusion masses" found in the muscles of monkeys during the early stages of experimental poliomyelitis (*cf.* Figs. 3 and 4).[2] Coincident in time with the projection of these masses from the hypolemmal axon out into the muscle fiber, there was a corresponding depletion or exhaustion of the epilemmal and hypolemmal motor axons resulting in loss of innervation of the voluntary muscle. During the end stage of shock, 5458 end plates were denuded from 6000 epilemmal axons counted

[11] Carey, E. J., *Anat. Rec.*, 1941, **81**, 393.

in the gastrocnemius muscle from a rat that had been in profound shock for 14 hours. This is comparable to the denudation of end plates by experimental poliomyelitis (*cf.* Figs. 5 and 6)[2] and injection of lactic acid locally in the zone of motor innervation of skeletal muscle (*cf.* Figs. 2 to 5).[4] The number of epilemmal axons denuded of end plates or possessing pathologic end plates varied in different fibers within a single muscle as well as in different muscles of the body. The intercostal muscles from the rat in shock for 14 hours were characterized in many places by large masses of axonic material that had flowed out from the end plates into the muscle fibers as well as between them. This axonic substance appeared to have drained right through the terminals of the motor nerves and to have left them in a depleted condition. In the final stage of irreversible shock, stimulation of the sciatic nerve failed in many instances to cause a response in the gastrocnemius and certain other muscles. Many muscles in the end stage of shock were in a greatly reduced state of irritability or had lost completely the capacity to respond to direct electric or mechanical stimulation. Many muscle fibers whose irritability was reduced or lost had so-called Zenker's waxy and granular degeneration with irregularities and loss of cross striations, increased visibility of pycnotic nuclei, and perivascular infiltration of leucocytes. Some of the intramuscular capillaries and venules were dilated and contained agglutinations of red blood cells. In other locations these vessels were collapsed and devoid of cellular constituents. Prolonged anesthesia with nembutal produced retraction and accumulation of Kühne's granules of the majority of the end plates, whereas ether expanded many of the end plates depleted of Kühne's granules. The changes produced by anesthesia are not as pronounced as those produced by irreversible hemorrhagic shock. In shock there is, in addition to those changes in the end plates produced by anesthesia, complete granulation, liquefaction, and projection into the muscle of axonic material, of many of the motor end plates. This produces loss of structure and function of the voluntary motor innervation at the neuromuscular junction.

Comments. "Chromodacryorrhea" or the shedding of so-called bloody tears, is produced in rats by injecting dacryorrhetin, a compound prepared from muscle,[12] and by acetylcholine.[13] Acetylcholine when injected into rats brings about an excessive secretion of saliva, intense lacrimation, and the appearance of a material in the tears which greatly resembles blood. This material, an excretory product of the Harderian gland, has been identified spectroscopically as a mixture of protoporphyrin and coproporphyrin. Tashiro proposed that the excretion to which he applied the name "chromodacryorrhea" be used as a biological assay for acetylcholine. A study has been made on the removal of acetylcholine by cholinesterase injections and the effect thereof on nerve impulse transmission[14] to the muscles of the iris. The relationship of dacryorrhetin[12] to adenosine triphosphate[15] is unknown. If the so-called bloody tears were produced by acetylcholine the phenomenon should have appeared early in shock. The fact that the bloody tears appeared in the terminal stage of shock suggests that some breakdown product accumulates in the blood stream during anerobic muscle metabolism. Additional evidence is needed to clear this point. The nervous exhaustion theory,[16,17] prevalent during the Civil War period, recently has been revived.[18–21] Possibly when the pathologic

[12] Tashiro, S., and Stix, H., *Biol. Bull.*, 1935, **64**, 327; Tashiro, S., *Proc. Am. Soc. Biochem.*, 1937, **8**, 98.

[13] Freud, J., *Acta Brevia Neerl.*, 1933, **3**, 159; Selye, H., *Canad. Med. Assn. J.*, 1937, **36**, 200; Tashiro, S., *Kongressbericht. II des XVI Internat. Physiologenkongress*, 1938, 46.

[14] Mendel, B., and Hawkins, R. D., *J. Neurophysiol.*, 1943, **6**, 431.

[15] Green, H. N., *Lancet*, 1943, **2**, 147; Bielschowsky, M., and Green, H. N., *Ibid.*, 1943, **2**, 153.

[16] Michell, S. W., *New York Med. J.*, 1866, **2**, 321.

[17] Gross, S. D., *System of Surgery*, Phila., Lea, 1872, v. I, p. 426.

[18] O'Shaughnessy, L., and Slome, D., *Brit. J. Surg.*, 1935, **22**, 589.

[19] Lorber, V., Kabat, H., and Welte, E. J., *Surg. Gyn. and Obst.*, 1939, **68**, 278.

[20] Phemister, D. B., and Schachter, R. J., *Ann. Surg.*, 1942, **116**, 610.

mechanism of shock is completely known the voluntary neuromuscular factor may be found closely related to the production of toxins[22-25] and to local fluid loss from capillaries.[26-28]

Summary. The limited evidence in this paper supports the claim that hemorrhagic shock profoundly alters the morphology of the motor end plates and finally produces loss of structural innervation of many muscle fibers in a single voluntary muscle. This histologic change is highly irregular in the different muscles of the rat, therefore large numbers of specimens from different muscles were teased. Gold staining masses of axonic materials drain out into and between the muscle fibers coincident in time with the loss of motor innervation due to the increased permeability of the end plates. The epilemmal axons, exhausted of their substances, are in many places likewise denuded of their hypolemmal end plates. There is therefore a real anatomic breakdown of many motor end plates and histologic alteration of certain skeletal muscles in hemorrhagic shock.

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21 Eversole, W. J., Kleinberg, W., Overman, R. R., Remington, J. W., and Swingle, W. W., *Am. J. Physiol.*, 1944, **140**, 490.

22 Dale, H. H., *J. Exp. Path.*, 1920, **1**, 103.

23 Cannon, W. B., *Traumatic Shock*, D. Appleton and Co., N.Y., 1923.

24 Bayliss, W. M., *J. Physiol.*, 1918, **52**, 17.

25 Moon, V. H., *Shock and Related Capillary Phenomena*, Oxford Univ. Press, N.Y., 1938.

26 Blalock, A., *Arch. Surg.*, 1931, **22**, 610.

27 Harkins, H. N., *Surgery*, 1941, **9**, 231.

28 Freeman, N. E., Freedman, H., and Miller, C. C., *Am. J. Physiol.*, 1941, **131**, 545.

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Cutaneous Application of Ethinyl Estradiol in Alcohol.

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It has been shown by Zondek[1] and Moore[2] that the estrogens, estrone and estradiol benzoate, respectively, were absorbed through the skin when administered by inunction in any oily vehicle. Later Zondek[3] showed that percutaneous absorption was even more effective when estrone was placed in an alcoholic solvent.

With the introduction of ethinyl estradiol, the new synthetic ester of the natural steroid estrogen estradiol in which the hydroxyl of carbon 17 is replaced with an ethine group,[4] it was considered advisable to test for percutaneous absorption of this hormone, in an alcoholic solution, on the pituitary, gonads, reproductive tract and body growth of the albino rat.

Experimental procedure and methods. Ethinyl estradiol‡ was dissolved in absolute alcohol to facilitate evaporation after application. One calibrated drop, or 2 in a few

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1 Zondek, B., *Klin. Wschr.*, 1929, **48**, 2229.

2 Moore, Carl R., Lamar, Jule K., and Beck, Naomi, *J. A. M. A.*, 1938, **111**, 11.

3 Zondek, G., *Lancet*, 1938, **1**, 1107.

4 Inhoffen, H. H., and Hohlweg, H., *Naturwissenschaften*, 1938, **6**, 96.

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