TABLE I.

Glycoside	Dilution, × 1000	Esterase	mm³ CO_2/30 min Control	with glycoside	% change
Digitoxin	1:5,000	Eel	130.69 (3)*	132.14 (3)*	+ 1.1
"	1:1,000	"	134.60 (3)	152.64 (3)	+13.4
"	1:1,000	Serum	165.59 (3)	178.64 (3)	+ 7.9
"	1:100	Eel	109.92 (3)	124.20 (3)	+13.0
"	1:10	Serum	196.55 (3)	191.08 (3)	— 2.8
Ouabain	1:250	Eel	73.00 (1)	83.40 (1)	+14.2
"	1:250	Serum	142.80 (1)	150.48 (1)	+ 5.4
"	1:100	Eel	91.44 (3)	88.85 (3)	— 2.8
"	1:10	"	73.17 (3)	81.64 (3)	+11.6
"	1:10	Serum	188.64 (3)	173.79 (3)	— 7.9
Strophanthin	1:500	Eel	98.59 (2)	85.54 (3)	—13.2
"	1:500	Serum	181.06 (3)	189.04 (3)	+ 4.4
"	1:10	"	186.72 (3)	208.99 (3)	+11.9
Lanatoside C.	1:40	Eel	73.70 (2)	81.10 (3)	+10.0
" "	1:40	Serum	220.74 (3)	221.87 (3)	+ 0.5

* The numbers in parentheses indicate the number of determinations and the value shown is the average.

The flasks were equilibrated with a mixture of 5% carbon dioxide and 95% nitrogen, and allowed to reach thermal equilibrium in the bath at 37°C. After an initial reading the vessels were tipped and readings made at 10-minute intervals for a period of one to 2 hours. Controls were run simultaneously in each experiment.

Using the straight line portion of the hydrolysis curve over a period of one-half hour, the results were expressed as the number of mm³ of carbon dioxide liberated per 30 minutes.

Table I shows the results of 15 experiments on the effect of the different dilutions of the various glycosides on both serum and eel esterase. In general there is no significant alteration in the activity of either enzyme as a result of the addition of digitalis. An average increase for all the glycosides and all dilutions of 3.1% for eel esterase and 2% for the serum esterase took place but in view of the individual variations these differences cannot be regarded as significant.

The serum esterase activity of 2 cats was determined at a time when the animals were near death as a result of intravenous doses of digitoxin, given at 5-minute intervals. One cat received 0.444 mg/kg over a period of 97 minutes, and the other 0.675 mg/kg of digitoxin in 135 minutes. The esterase activity of the serums taken just prior to death in each case was not significantly different from the values obtained before digitalis.

Summary. These results confirm the observation that the cardiac slowing by digitalis is not due to inhibition of cholinesterase.

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Exaggerated Discharge of Neurosomes into Spastic Muscle by Heat Reflex.*

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Morphologic evidence of the transmission of nervous substance into the myoplasm of spastic muscle from the motor end plates has not been demonstrated. Neurosomes were first described as "auriphilic inclusion masses"

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in the abnormal muscles of the monkey[1] and man[2] during the early stages of poliomyelitis; after injection of lactic acid[3] into rat's muscle; and during the onset of chemical,[4] electric,[5] hemorrhagic,[6] traumatic,[7] and thermal[8] shock. The purpose of this study is the morphologic demonstration of neurosomes discharged from the large motor end plates into the muscles of chameleons immediately after their spastic or cramp-like response to the reflex action of heat applied to the skin.

Methods. The following easily reproducible experiments were made. Ten chameleons (*Anolis caroliniensis*) 3 to 5 g were decapitated. The 20 biceps femoris muscles were excised and used as controls after they were subjected to various histologic technics, viz., gold,[5] Bielschowsky silver, methylene blue, osmic acid, sudan III, Herxheimer's alkaline scarlet red in both teased and sectioned tissue. The freshly teased muscles, frozen longitudinal and cross sections were observed.

Additional control experiments were also performed by the following method. The spinal cord was destroyed from the base of the tail to a level 2 cm cephalad by the pithing method with a teasing needle in 40 unanesthetized chameleons. Both hind extremities were in a state of flaccid paralysis after pithing the cord. There was no reflex response of any hind limb upon amputation of a toe with a pair of scissors. The caudal one-half of each animal was then immersed in water 70°C for one second after the following time intervals subsequent to pithing the spinal cord: 6 animals immersed immediately, 8 animals immersed after 2 hours, and 26 animals after 8 hours. There was no reflex response of the muscles of the hind limbs to the thermal stimulus applied to the skin. The tails of the immersed animals with the intact caudal part of the spinal cord, however, manifested reflex responses by rapid lateral and circular movements. The 80 hind limbs were skinned and excised immediately after immersion of the animals. The biceps femoris muscles were then subjected to the gold technic.

Fifty living unanesthetized and unskinned chameleons were vertically immersed with the hind limbs, in an extended position, in water 70°C over the caudal one-half of their bodies for 1 second. Forty chameleons had immediate extensor spastic paralysis of both hind legs; 10 were not visibly paralyzed. The 50 animals were divided into the 4 following groups: (1) 10 of the spastic animals were decapitated immediately; (2) 10 spastics were decapitated after 2 hours; (3) 10 spastics and the 10 non-spastics were decapitated after 4 hours; (4) 10 spastic animals were allowed to live 24 hours at which time 8 had fully recovered, 2 still had rigidity of both hind limbs; these 10 animals were decapitated. The 100 bilateral biceps femoris muscles were excised from the 50 experimental animals and subjected to the same histologic methods as the 20 muscles from the 10 normal controls.

Results. Both hind limbs of 40 of the 50 chameleons immersed in water 70°C, 1 second, immediately had spastic paralysis. This was dominantly a reversible rigor or reflex cramp of the muscles because 8 of 10 animals fully recovered after 6 to 24 hours. The green color of the immersed part of the skin became dark brown.

The relatively normal motor end plates in the 10 decapitated control chameleons had a mean for the length of 1000 end plates of 87.6 μ and for the width of 32.9 μ. Some of the end plates were retracted (Fig. 1) with lack of definition between the terminal axons and the granules of Kühne. They were frequently associated with few coarse cross striations. The expanded end plates (Fig. 3) had a decrease in the granules of Kühne and were associated with many fine cross striations.

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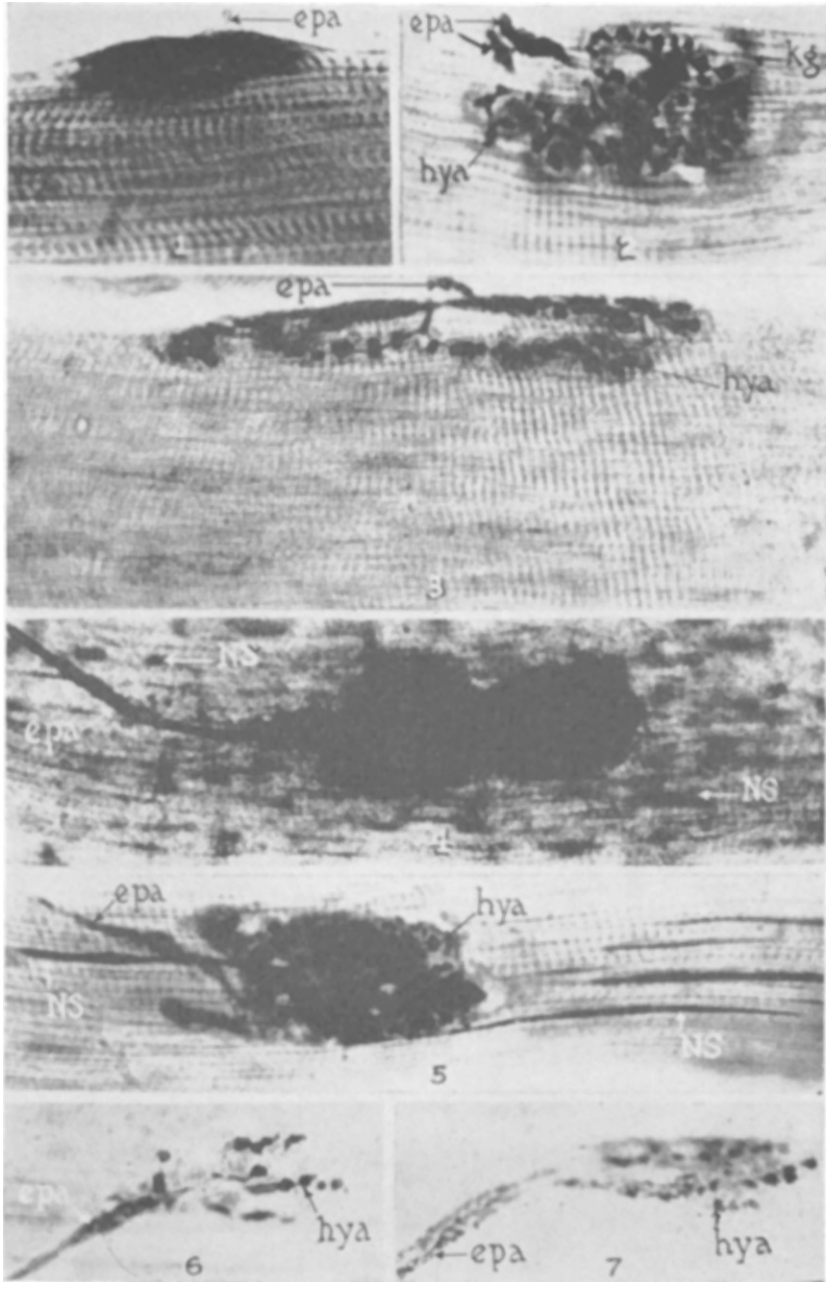

FIG. 1 to 7.
Photomicrographs, normal innervation, Fig. 1 to 3, biceps femoris muscle, chameleon, and after thermal trauma, Fig. 4 to 7; epa, epilammal axons; hya, hypolemmal axons; Kg., Kühne's granules; NS, neurosomes; gold chloride, teased whole muscle fibers. × 500.

Intermediate stages were found (Fig. 2) in which both the terminal axons and the granular sole of Kühne were clearly defined. This granular rim varied from 1 to 30 μ in width and either gradually or suddenly blended with the dark anisotropic bands of the cross striations. These cross striations were absent around some end plates. They were replaced

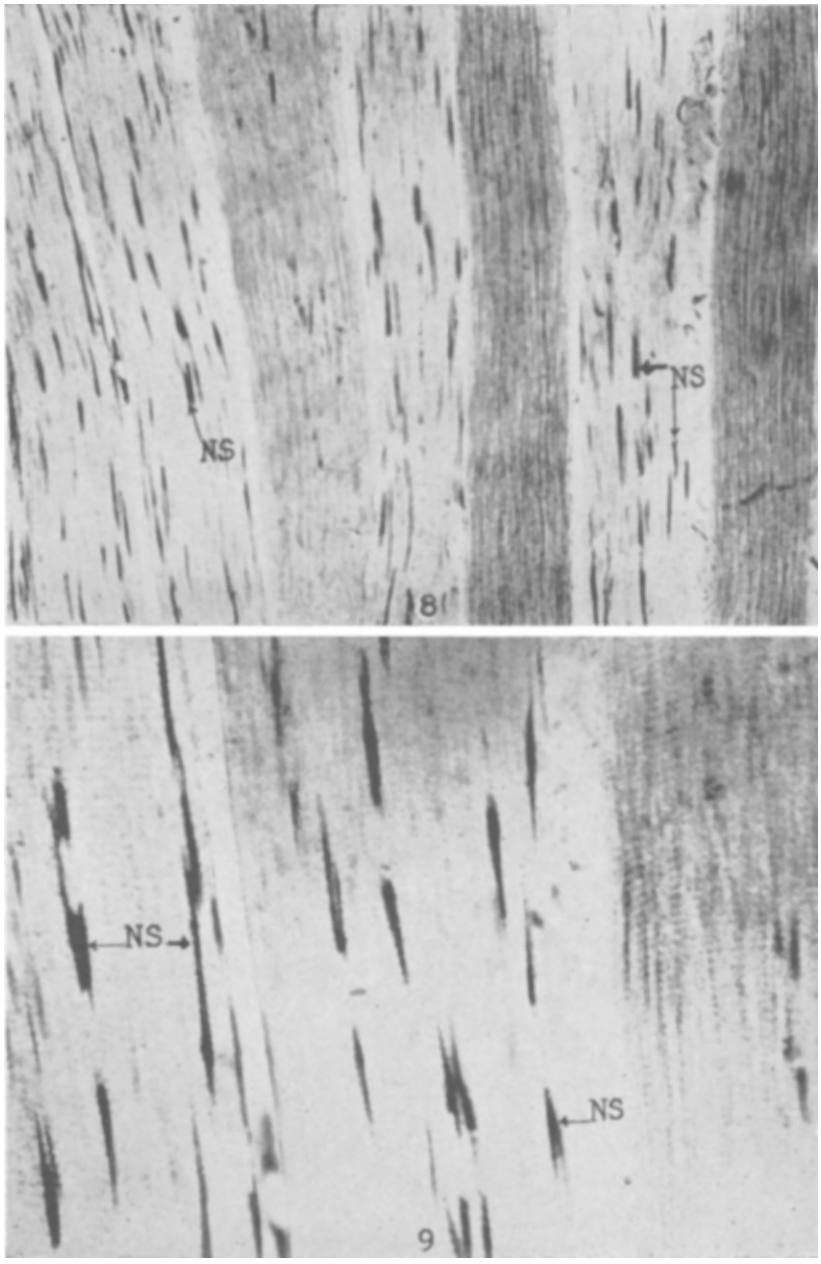

FIG. 8 AND 9.
Photomicrographs, biceps femoris muscle, chameleon, 2 hours after thermal trauma, 1 sec. duration of the skin. Neurosomes (NS.) dispersed in many muscle fibers; gold chloride, teased whole muscle fibers; Fig. 8, × 200; Fig. 9, × 500.

by a diffuse arrangement of granules. The retracted end plates (Fig. 1) had an increased capacity for gold; the expanded ones (Fig. 3) a decreased capacity.

The sequence of the morphologic effects of the reflex action of heat applied to the skin upon the motor end plates were the following: (1) increased capacity of the end plates for gold (Fig. 4, hyperchrysophilia) to the point of complete lack of definition between the

terminal axons and their transformation into the granules of Kühne; (2) exaggerated discharge of neurosomes or gold-impregnated bodies from the motor end plates into the myoplasm of the skeletal muscle fibers (Fig. 4 and 5, NS., neurosomes; axonorrhea); (3) progressive depletion to the level of complete structural exhaustion of the terminal axons of their substances with an affinity for gold (Fig. 6 and 7, hypochrysophilia; achrysophilia).

The neurosomes were found in 1 of the 20 relatively normal control muscles. They were present in a variable number of muscle fibers in 29 (Fig. 4 and 5, 8 and 9 NS.) of the 40 muscles of the experimental animals in Groups 1 and 2. Some of the muscle fibers had as high as 25% of their content composed of neurosomes intensely impregnated with gold. Some of the neurosomes were opaque, others were cross striated and clearly defined from the lightly impregnated cross striations of the muscle fiber. Neurosomes were found in variable amounts in 7 of the 20 spastic muscles and 3 of the 20 non-spastic muscles of the animals in Group 3. They were likewise distributed in a localized manner in 2 of the 16 fully recovered muscles and in 1 of the 4 spastic muscles of Group 4. These neurosomes, therefore, were greatly increased in frequency over that of the normal during the first 2 hours after heat trauma applied to the skin. Their disappearance appeared to be a function of the time interval after trauma and recovery of the animals from the reflex muscle rigor.

Neurosomes were found in scattered muscle fibers in 3 of the 12 muscles excised immediately after pithing of the spinal cord and immersion of the animals. The muscles of the hind legs underwent vigorous contraction during destruction of the spinal cord. Neurosomes were present in local areas in 1 of the 16 paralyzed muscles excised 2 hours after pithing of the spinal cord, at which time the animals were immersed. Neurosomes were also found in 1 of the 52 paralyzed biceps femoris muscles excised 8 hours after destruction of the spinal cord. These neurosomes were, therefore, slightly increased in frequency over that of the normal number in muscles excised immediately after pithing of the spinal cord and immersion of the animal. This increase of neurosomes was probably incident to the augmented neuromuscular activity associated with the traumatic destruction of the spinal cord. The neurosomes, however, were greatly decreased in frequency, under that of the normal number, in the paralyzed biceps femoris muscles excised 8 hours after destruction of the spinal cord. The great reduction in the number of neurosomes after spinal cord destruction was especially striking in comparison to that found in the spastic muscles of immersed animals with their spinal cords intact.

Neurosomes varied in morphology from the normal fine mantle composed of granules 0.5 to 2 μ in diameter of the sole of Kühne (Fig. 2, Kg) to oblong and fusiform bodies 10 to 1000 μ in length (Fig. 4, 5, 8, and 9, NS). There were droplets and vacuoles 5 to 35 μ in diameter (Fig. 5) in direct anatomic continuity with the terminal axons of some end plates. Some neurosomes were shaped like arrow heads and others had elongated irregular oblong forms. These dense neurosomes quickly underwent granulation, the granules of which became aligned into cross striations. During the first 2 hours after immersion some of the greatly elongated streamers resembled the ultraterminal non-medullated branches projected from the axons of the end plates observed infrequently in some control animals after decapitation. The ends of these ultraterminal branches varied in shape, viz., enlarged knobs, vacuoles, or gradually became attenuated into invisibility by incorporation into the myoplasm.

The neurosomes were inconstant in their capacity for gold, silver, osmic acid, sudan III, and scarlet red. With lipoidal stains the liposomes were spherical, oval, or fusiform in shape and varied in size and staining capacity. They had the appearance of non-nucleated fat droplets. They varied in refractile properties. Some large neurosomes had a myelin figure of a central maltese cross when observed with polarized light. When they were initially discharged during the first 2 hours after reflex action of thermal trauma applied to the skin, they had increased capacity for the above indicators. After the neurosomes had become incorporated into the myoplasm, they were

found for a time (4 to 24 hours after immersion) in some muscle fibers as fine, transversely aligned granules but in others they had completely disappeared.

Many of the muscle fibers had an increase of thick, dark, longitudinal myofibrils and a decreased prominence of the cross striations. In others, the cross and longitudinal striations completely disappeared and were replaced by granules or hyaline masses comparable to Zenker's waxy changes in muscle as well as abnormal waves of contraction. These were reversible waves because they were absent after recovery. The nuclei were frequently pycnotic especially in those fibers with an abundance of neurosomes. The pathologic changes in the myoplasm appeared to be in direct proportion to the number of neurosomes present during the first 2 hours after immersion of the animal. From 4 to 24 hours after immersion, in the 4 muscles of the 2 chameleons that remained spastic, there was progressive depletion of the end plates (Fig. 6 and 7), dissolution of neurosomes, and a more extensive pathologic change of the muscle than during the first 2 hours. During this late period after immersion, the muscular capillaries in many places were dilated, contained clumps of red blood cells, and had perivascular edema.

Discussion. The term *neurosomes* is herein defined as ephemeral, pleomorphic, and lipoidal bodies discharged into the myoplasm of striped muscle from superpermeable terminal axons of motor nerve plates: these neurosomes are composed of granules, droplets, vacuoles, or elongated streamers that vary in size and staining capacity for gold, silver, and lipoidal stains.

The current confusion regarding the true structure of the neuro-myoplasmic continuum composed of inconstant myofibrillæ, cross striations, sarcoplasmic granules, and the pleomorphic neuromuscular apparatus has been emphasized by Cobb[9] and Hinsey[10], respectively, and by Jordan,[11] Hines,[12] Tower,[13] Denny-Brown,[14] Boeke,[15] Wilkinson,[16] Murray,[17] and many others.

The relationship or identity of neurosomes with the interstitial granules of Kölliker[18] and the liposomes of Albrecht[19] is now under investigation. The experimental, tinctorial, and chemical methods used by Bell[20] to increase, decrease, and identify liposomes in muscle are precisely those that accentuate, eliminate, and identify the discharge of neurosomes from the motor end plates. In the past, the study of liposomes in muscle has not been correlated with the structural and staining changes of the motor end plates and those in striped muscle. When the histologist has supplied the needed data of the structural variations of the neuromuscular apparatus the pathologist will have criteria with which evaluations may be made of early changes of morphology involved in the onset of shock due to multiple extrinsic and intrinsic factors as well as of the so-called fatty metamorphosis of muscle. Evidence now at hand supports the above statements in their application to cardiac as well as skeletal muscle.

Summary. The limited experimental evidence presented tends to support the statement that the reflex effects of heat applied to the skin are associated with the exaggerated discharge of ephemeral, pleomorphic, and lipoidal neurosomes from the motor end plates into spastic or cramp-like muscle. The neurosomes have a variable affinity for gold, silver, and lipoidal stains. There are characteristic instantaneous changes in the morphology of

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the motor end plates and myoplasm in response to the reflex effects of thermal trauma applied to the skin.

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15113 P

Studies on Mode of Action of Streptomycin. I. Effect of Culture Media.

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In studying the mode of action of penicillin on bacteria, Hobby, Meyer, and Chaffe[1] and Lee, Foley, and Epstein[2] observed that multiplication must take place to get killing action and that the more rapid the growth of bacteria the greater the rate of destruction. Waksman and Woodruff[3] found the reverse to be true in the case of actinomycin and streptothricin. With these two antibiotics it was more difficult to inhibit bacteria on a good medium than it was on a poorer medium. The study reported here is an attempt to determine the action of streptomycin on bacteria and to see if this action was similar to either the action of penicillin or of actinomycin and streptothricin.

The streptomycin was prepared at the University of Illinois in the laboratories of H. W. Anderson and purified and standardized in the laboratories of H. E. Carter.[4,5] The unit used is practically the same as the Waksman dilution unit.[6]

Eberthella typhosa (Hopkins strain) and *Staphylococcus aureus* (FDA 209) were used as the test organisms, and were tested against the streptomycin according to the following procedure.

The following media were compared: (1) nutrient broth, (2) nutrient broth diluted with an equal amount of water, and (3) brain heart infusion. Because the presence of phosphate and NaCl might affect the action of streptomycin it was thought not advisable to use Difco brain heart infusion, but instead the brain heart infusion was made according to the following procedure. Five hundred g of ground beef heart were infused with 1000 ml of water and 500 g of beef brain were similarly treated. These infusions were separately filtered through gauze, heated to boiling and filtered again. The medium was made from these infusions using the following formula:

Beef heart infusion	75 ml
Beef brain infusion	25 "
Peptone (Bacto)	1 g
Glucose	0.2 "

Adjusted to pH 7.0 and sterilized.

Streptomycin was added in varying amounts to tubes containing 15 ml of each of these media and *Eberthella typhosa* and *Staphylococcus aureus* were added. Twenty-four-hour broth cultures of the organism were diluted with twice their volume of sterile water in order to obtain an inoculum which would not be too heavy and 0.1 ml of this suspension was used as the inoculum. Immediately thereafter a bacterial plate count was made on each tube

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