been chosen as end-points, namely: onset of anoxic T-wave; cardiac slowing of 18 beats/min.; increase in frequency and also flattening of the electrical activity of the cortex; and onset of isoelectricity of the EEG. The onset of these, abstracted from the continuous records, are plotted on the straight lines representing the rates of injection. The points for each variety of end-point lie on curved lines. The complete curves for one representative experiment (Cat No. 155) are presented (Fig. 1) as well as one composite curve containing the average values for 10 animals (Fig. 2). It will be noted that while the various effects of NaCN occur serially close in time at high rates of injection, at slower rates of injection they are disproportionally spread out in time.

Discussion. It is not surprising that the oxygen tension is inconsistent when both the consumption and the supply are variably decreased.

If detoxification of NaCN in the body proceeded at a constant rate, the plots of the end-points of any variety would fall on a straight line. The observed ascending curves being convex upward, indicate that the rate of detoxification increases with the concentration—as it would if it followed the law of a mass action. The clinical correlate of this concept is that a greater margin of safety obtains at slower rates of injection and for higher end-points. Thus in Fig. 1 there is an interval of 3 minutes between EEG isoelectricity and EEG flattening when NaCN is injected at 73 μg/kg/min. and an interval of 7 min. at 38 μg/kg/min. At slower rates the interval increases greatly and at 25 μg/kg/min. isoelectricity never occurs.

As the rate of injection is increased so as to approach the injection of the whole dose in 7 seconds, new factors begin to determine the time of appearance of the end-points. These include not only circulation time and time for diffusion into the tissues, but the time for the alteration of the tissue and the time for the development of the particular phenomenon as well as other things at present unknown. Any attempt to extrapolate from the curves for slow injection to the time of appearance of any sign after sudden injection is clearly unwarranted. It is important to note that these other factors determine delays too short to account for the observed curvature.

Summary. NaCN in dilute solution has been injected slowly at constant rates ranging from 7 to 100 μg/kg/min. The time and dose, at which several phenomena are first encountered, plot in ascending curves which are convex upward. This curvature probably indicates that detoxification of NaCN proceeds rapidly at higher concentrations.

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Effects of Use and Disuse on Nerve Endings, Neurosomes, and Fiber Types in Skeletal Muscle.*

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Pathologic neurosomes were first identified in the muscles of man[1] and monkey[2] during the early acute changes produced by poliomyelitis, and later in the muscles of rats and

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1 Carey, E. J., Massopust, L. C., Zeit, W., and Haushalter, E., *J. Neuropath. and Exp. Neurol.*, 1944, **3**, 121.

2 Carey, E. J., *Am. J. Path.*, 1944, **20**, 961.

chameleons during the onset of shock following hemorrhage,[3] heat,[4] trauma,[5] chemical action,[6] and histamine injection.[7] Suggestive evidence of the probable relation of the periodic secretion from the nerve endings to the granular and agranular muscle fibers, as well as to the clumping effect of DDT[8] on the neurosomes, had been demonstrated. The discharge of pathologic neurosomes into muscle during the early stages following nerve section[9] had likewise been shown, as well as the possible relation of the progressive loss of the normal, fine neurosomes to the gradual disappearance of the normal, dark and granular muscle fiber during atrophy. The purpose of this paper is the histologic demonstration of the giant fusiform neurosomes, discharged from nerve endings, retarded in rate of discharge, diffusion, and dissolution during the early stages of muscular atrophy of disuse following tenotomy of the innervated gastrocnemius muscle of the rat.

Methods. Under aseptic surgical technic, the Achilles tendon of the right gastrocnemius muscle was completely severed by transverse section from the calcaneus, and 3 mm of the distal end of the tendon were excised, in 150 white rats *(Mus norvegicus)*. The gastrocnemius muscle of the left leg was allowed to remain intact and used as a control of the effects of normal use. At 24-hour intervals the morphologic changes in the right gastrocnemius muscle were compared with the normally used muscle of the left side in each of 5 rats over a 30-day period. Segments of the control and experimental gastrocnemius muscle and sciatic nerve were simultaneously subjected to the same histologic technics. In 30 additional white rats, 2 were selected at 48-hour intervals following tenotomy. A ligature tied at the cut end of the muscle had at the free end of the ligature suspended weights that varied from 10 to 30 g depending upon the size of the muscle. The living muscle *in situ* was gradually restretched prior to excision and gold impregnation for 10- to 15-minute periods alternating with 5 minutes of rest for 3 to 5 hours.

The method of gold impregnation and teasing of whole muscle fibers, previously described,[1-9] was found superior to any other neurologic technic for the detection of the structural changes of the nerve endings in muscle. This method was checked against the current popular ones in which silver or methylene blue are used. The muscles were likewise stained with osmic acid, Sudan III, Sudan black, and Scharlach R, as well as with ordinary stains such as hematoxylin and eosine, after fixation with either formalin, Zenker's fluid, or other fixatives. Formalin produced chemical changes and altered critical features of the morphology of the neuromuscular apparatus. No alcoholic dehydrating agent was used in the gold method of teased whole mounts. The teasing of whole muscle fibers was a better technic than that of cutting the muscle into sections for observation of the whole neuromuscular apparatus and the anatomical relationships and changes of the epilemmal axon, hypolemmal axon, granules of Kühne, cross striations, and the granular and agranular muscle fibers. The following experimental observations will be confined to the structure of nerve endings and muscle revealed by the gold technic.

Results. Effect of normal use on nerve and muscle. The normal muscle observed after gold impregnation and in teased whole muscle fibers had narrow, dark, and coarsely granular muscle fibers (Fig. 1) scattered among others of various diameters which were either finely granular or relatively

3 Carey, E. J., Massopust, L. C., Zeit, W., Haushalter, E., and Schmitz, J., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 115.

4 Carey, E. J., Massopust, L. C., Haushalter, E., and Zeit, W., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 121; *Am. J. Path.*, 1946, **22**, 175.

5 Carey, E. J., Massopust, L. C., Zeit, W., Haushalter, E., and Schmitz, J., *J. Neuropath. and Exp. Neurol.*, 1945, **4**, 134; Carey, E. J., Massopust, L. C., Zeit, W., Haushalter, E., Hamel, J., and Jeub, R., *Am. J. Path.*, 1945, **21**, 935.

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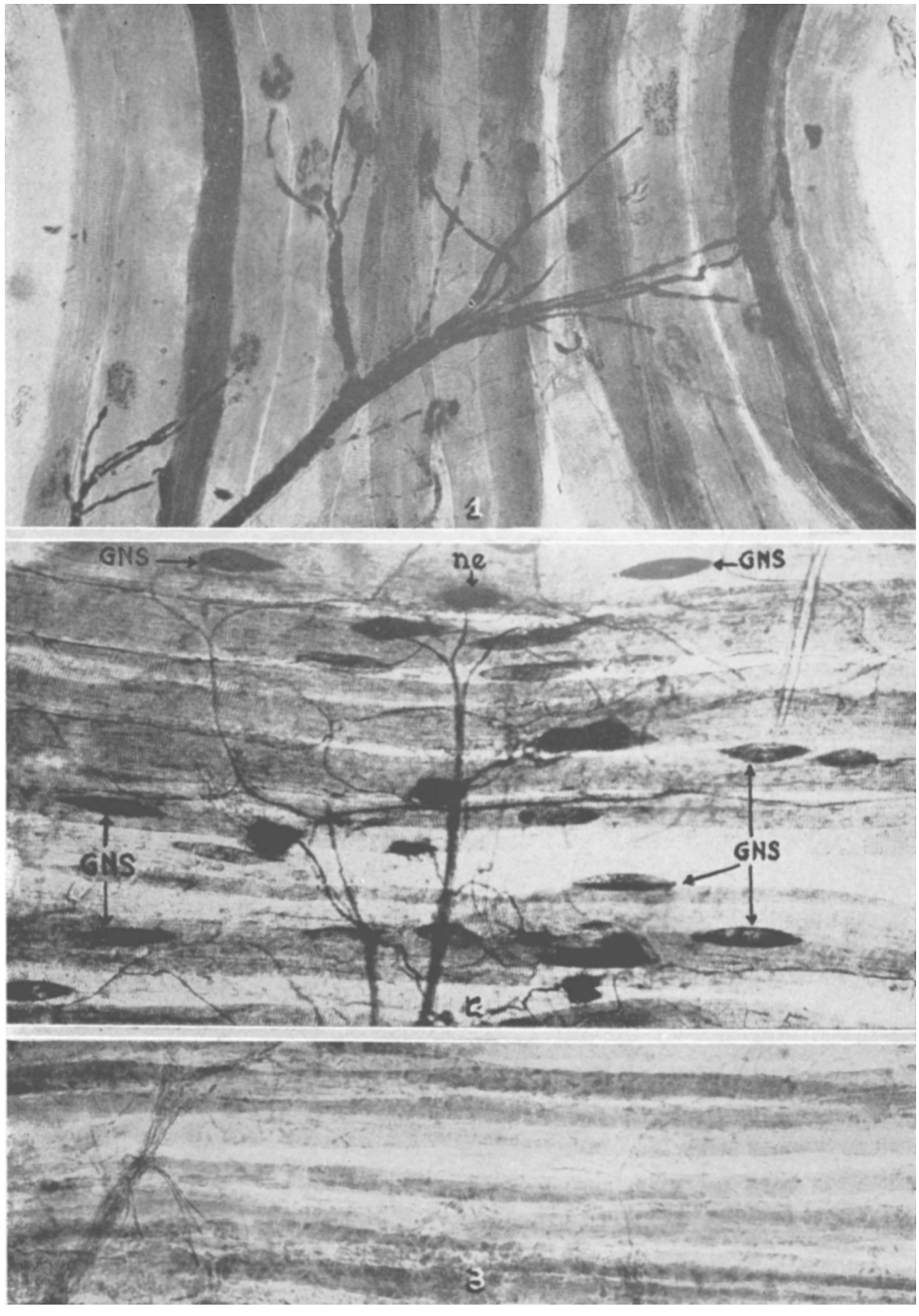

FIG. 1 TO 3.

Photomicrographs × 150. Normal innervation, Fig. 1; discharge of giant, fusiform neurosomes from end plates 15 days after tenotomy, Fig. 2; and atrophy of nerve endings and muscle 30 days after tenotomy, Fig. 3, whole, teased, gastrocnemius muscle fibers, white rat. Legend: ne, nerve endings; GNS, giant fusiform neurosomes. Gold chloride technic.

agranular and light (Fig. 1 and 4). These dark fibers were designated as hyperchrysophilous and the light ones as hypochrysophilous and achrysophilous. There were multiple gradations of affinity for gold between the extremes. The nerve endings were usually retracted and deeply impregnated with gold (Fig. 1) in the hyperchrysophilous, dark muscle fibers. In the hypochrysophilous fibers the nerve endings were usually expanded (Fig. 1) and had a decreased affinity for gold. The granules of Kühne usually formed a dense rim around the retracted nerve endings whereas there was a quantitative diminution of these granules, to the point of complete depletion, around the extended branches of the expanded nerve endings produced by neuroprotoplasmic streaming. In a differential count of 5000 nerve endings the retracted endings varied in length from 20 to 40 μ and the expanded endings from 40 to 60 μ.

The normal muscle in cross section (Fig. 4) had relatively narrow fibers with coarse granules or neurosomes and the wide fibers contained medium-sized and fine granules. There were variations in the size of the granules, however, in the fibers of different diameters. In other locations in this same muscle, some of the wide fibers were relatively agranular. The axon, nerve endings, granules of Kühne, and granules in the muscle fiber, all had the same reaction to gold. The size and distribution of the granules were assumed to be related to the different degrees in the process of hydrolysis after the neurosomes were discharged into the muscle.

Effect of tenotomy on nerve and muscle. There was a progressive loss of the differential types of muscle fibers following tenotomy (Fig. 2 and 3). The characteristic normal dark type of granular muscle fiber was gradually lost until, on the 30th day (Fig. 3) following tenotomy, it was very infrequently found. There was, likewise, a great depletion (Fig. 3) of the nerve supply. In some places the hypolemmal axons of the nerve endings were completely absent (Fig. 3) and all that remained were clumps of sole plate nuclei. The structural expression of the dark, coarsely granular muscle fiber (Fig. 1 and 4) was determined by the presence of the attached and normally functioning muscle with its innervation. The nerve endings became uniformly and deeply impregnated with gold and fusiform in shape (Fig. 2) between the 10th and 20th days following tenotomy in the rat. These fusiform nerve endings were moulded by the progressive shrinkage of the muscle fiber due to loss of muscle substance.

On the 15th day (Fig. 2) of disuse atrophy following tenotomy, there could be seen manifestations of various stages in the discharge of giant fusiform neurosomes from the oblong and spindle-shaped nerve endings which were hyperchrysophilous. From the 3rd to the 15th day following tenotomy, in certain places in the muscle, a progressive increase in the number of the giant neurosomes was found. From the 15th to the 30th day following tenotomy, there was a progressive decrease in the number of these giant neurosomes. The discharged giant neurosomes were irregularly scattered in the myoplasm. Some of these giant fusiform neurosomes were uniformly and deeply impregnated with gold. Some were light in the center and dark at the tapering ends. Others were light at the ends and dark in the center. Still others were very faintly impregnated with gold, and their contained granules were arranged in cross striations undergoing progressive alignment with the cross striations of the muscle fiber.

These fusiform neurosomes varied from 10 to 275 μ in length and from 4 to 60 μ through the widest transverse diameter. In some places there were small oblong and fusiform neurosomes similar, in morphology and staining reaction to gold, to those observed during the early stages after nerve section in denervated muscle.[9] The large fusiform neurosomes produced a streamlining effect (Fig. 5 and 6) on the cross striations of muscle during the process of migration from the nerve ending and dispersion in the myoplasm of the muscle fiber. Some of these neurosomes in transverse sections were immediately under the sarcolemma whereas oth-

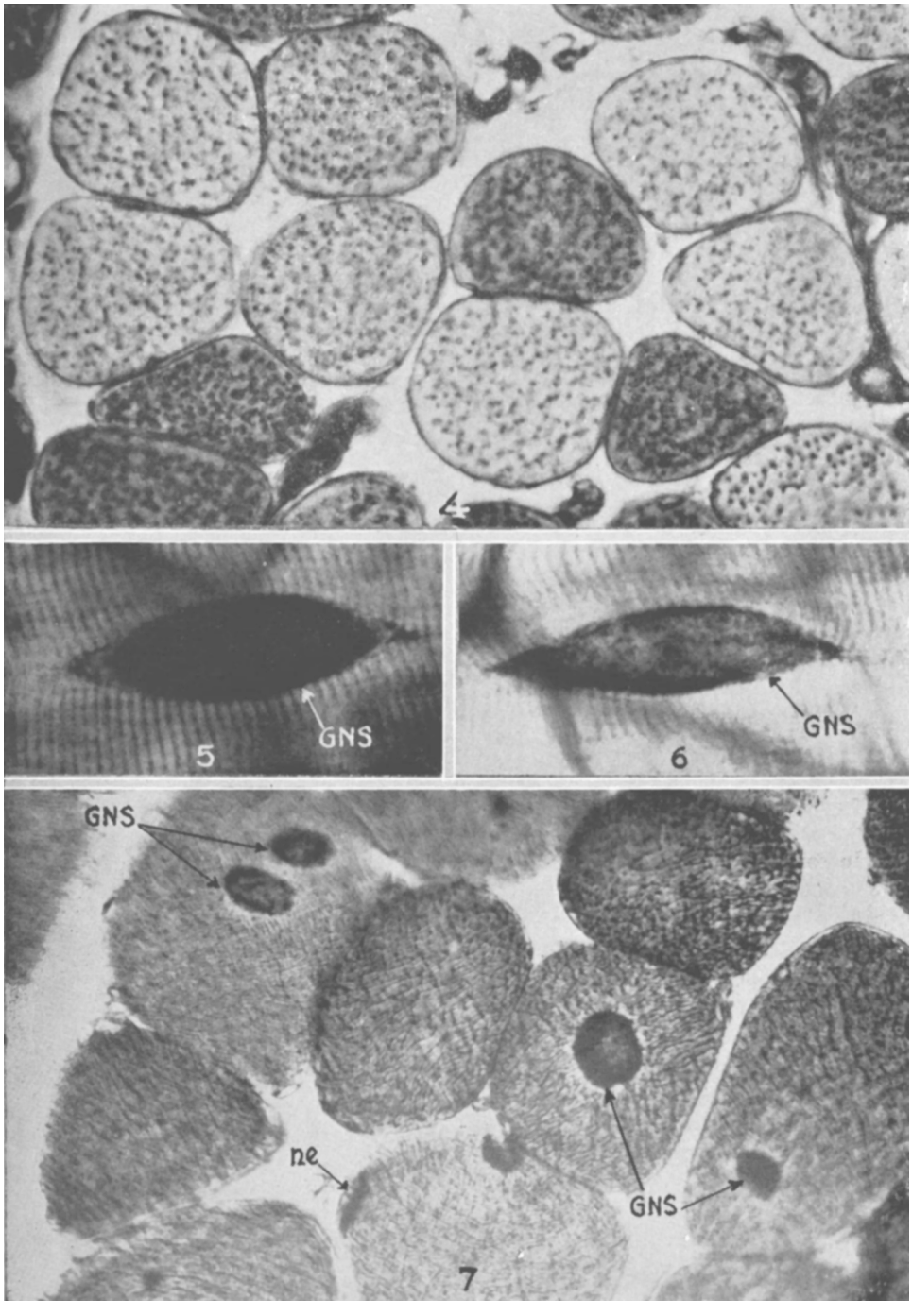

FIG. 4 TO 7.

Photomicrographs. Cross sections, normal, coarsely and finely granular muscle fibers × 400, Fig. 4; giant fusiform neurosomes in teased whole gastrocnemius muscle fibers × 600, Fig. 5 and 6; and cross sections of fiber containing giant fusiform neurosomes, gastrocnemius muscle, white rat, × 600, Fig. 7. Legend: ne, nerve endings; GNS, giant fusiform neurosomes. Gold chloride technic.

ers occupied the center (Fig. 7) of the muscle fibers. During disuse atrophy there was a progressive loss of the normal dark muscle fiber and of the normal coarse granules (Fig. 7) of the neurosomes. The granules were either very fine and dispersed, or aggregated into the giant fusiform neurosomes. In cross sections (Fig. 7) the giant neurosomes were either uniformly impregnated deeply with gold, or they had a light center or a rim of granules faintly reacting to gold.

The gastrocnemius muscle lost 30 to 50% of its weight, compared to the normal control, between 21 to 30 days after the tenotomy. There were individual variations in the rate of atrophy and in the morphologic changes of the neuromuscular apparatus and fibers of the gastrocnemius muscles. These variations were found not only in muscles from different animals examined after the same time interval following tenotomy, but also in different fibers in the same muscle. It was necessary, therefore, to survey great numbers of muscle fibers to detect the statistical trend of the morphologic changes.

The reestablishment of partial muscle stretch in some fibers of the muscle by regenerative attachment of the tendon to the subcutaneous tissue was a variable to be taken into consideration in evaluating the results. In some of the rats in our series the tendon of the muscle was freshly cut at 7-day intervals to eliminate the effects of partial reestablishment of stretch by union of tendon to subcutaneous tissue. The loss of normal muscle stretch determined the discharge of giant neurosomes. This was indicated by the total absence of the giant neurosomes in the series of 30 living muscles experimentally restretched *in situ* before excision and gold impregnation. Two muscles were restretched at 48-hour intervals until the 30th day after tenotomy.

Discussion. The basic mechanism in the physiology of use, increased use by exercise, and disuse, resulting, respectively, in muscle maintenance of health, hypertrophy, and atrophy of disuse and disease, is unknown. Young[10] stated that "the theories of the causes and nature of muscular atrophy are numerous but none is conclusive." This conclusion is held likewise by Carlson and Johnson.[11] The parts of the living body increase and decrease in size in proportion to the functional demand or use, but the essential underlying cause of these changes has remained an elusive one.

The hyperexcitability manifested by fibrillations may be prevented by quinidine, but Solandt and Magladery[12] observed that the denervated muscle continues to shrink in size. The slow, incoordinate activity of fibrillation, therefore, appears not to be the cause of the muscular atrophy. It has been demonstrated by Gutmann and Gutmann,[13] Hines,[14] Eccles,[15] and Solandt,[16] that the volume of denervated muscle may be fairly well maintained for a certain period by appropriate electrical exercises. Evidently, periodic optimum tension of traction and contraction (work) of muscles anchored to their attachments is necessary for muscle maintenance during health. Suggestive evidence was demonstrated previously that an optimum periodic tension of differential growth is necessary for the genesis of both smooth and skeletal muscle.[17] Evidence was also demonstrated that anatomic and experimental dampers to the lateral expansion of the stretched muscle fibers produces a replacement of muscle by fibrous tissue.[18]

The normal tension, or stretch, and work of the intact muscle attached to origin and

10 Young, J. Z., *Lancet*, 1946, **2**, 109.

11 Carlson, A. J., and Johnson, V., *The Machinery of the Body*, University of Chicago Press, Chicago, 1937, 620 pp.

12 Solandt, D. Y., and Magladery, J. W., *Brain*, 1940, **63**, 255.

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16 Solandt, D. Y., de Lury, D. B., and Hunter, J., *Arch. Neurol. Psychiat.*, 1943, **49**, 802.

17 Carey, E. J., *J. Gen. Physiol.*, 1920, **2**, 357; *J. Gen. Physiol.*, 1920, **3**, 61; *Am. J. Anat.*, 1921, **29**, 341; *J. Morphol.*, 1922, **37**, 1.

18 Carey, E. J., *Am. J. Anat.*, 1936, **59**, 89.

insertion, appear to be necessary for the normal rate of discharge of granules from the motor end plates. This secretory process from the nerve endings may be slowed down by disuse of the innervated gastrocnemius muscle following tenotomy. By tenotomy, the normal stretch or tension of the muscle is destroyed. The lax muscle fibers released from one attachment are analogous to the broken strings of a violin. The detached strings are incapable of normal vibratory response because of loss of tone or tune. The rate of discharge of neurogenic substance into the muscle appears to depend upon the reciprocal interaction between nerve and muscle. The normal mechanical tension of the attached muscle fibers appears to determine the normal periodical flow of neurogenic substances into the muscle. The normally attached muscle fiber appears to act like an alternate pressure and suction chamber upon the nerve ending. This nerve ending appears to be a biological jet valve or ejector of the neurogenic secretion. Under normal conditions the rate of discharge, diffusion, and disappearance of neurosomes is excessively rapid. The release of normal muscle stretch by tenotomy decreases the demand, and slows down the rate resulting in accumulation of the neurogenic discharge into the abnormally flaccid muscle fibers.

Evidence now at hand supports the statement that the changes of fatty metamorphosis, Zenker's hyaline degeneration, the pathogenesis of dystrophy and of poliomyelitic muscle, are closely related to the pathology of the neuromuscular apparatus and alterations in the secretion of neurosomes into muscle. In the past, many of the fine physiologic neurosomes have been identified variously as the interstitial granules of Kölliker,[19] the J and Q granules of Holmgren[20] and the liposomes of Albrecht[21] and Bell.[22]

Evidence[1-7] had been accumulated and presented that the full fractional contraction, the relative relaxation, and the onset of contraction of functional activity, all were correlated with the types of nerve endings and muscle fibers. The dark, granular muscle fibers were proportionately increased in number by magnesium sulphate[9] and by agents that inhibited cholinesterase in its hydrolytic action on acetylcholine, such as thiamin chloride, atropine, curare, prostigmine, and ergotmine.[23] In high concentrations (0.25 to 0.5%) these chemicals injected into the living muscle increase proportionately by 25 to 50% the number of the dark granular muscle fibers.[23] On this incomplete evidence it is assumed that some of the granules in the dark muscle fibers are composed, at least in part, of acetylcholine.

The evidence presented in this paper supports the principle of the *Double Dependence* of nerve and muscle proposed by Young.[10] He stated that muscle receives its stimulation from nerve and exercises constraint against other muscles or outside forces. Muscle will atrophy if given too little direction from above, as after total denervation or isolation of lower from upper neurones; it will also atrophy if it is left relaxed by tenotomy and, therefore, cannot contract against resistance. We have observed, also, that disuse of the relaxed and attached gastrocnemius muscle, in the rat, by fixation of the whole limb in a cast produces changes in the neuromuscular apparatus similar to the effects of tenotomy.[24]

Summary. The limited experimental evidence presented in this paper tends to support the following statements:

The normally used and innervated gastrocnemius muscle of the white rat is characterized histologically by dark, coarsely granular and light, finely granular and

[19] Kölliker, A., *Z. f. Wissensch. Zool.*, Leipzig, 1857, **8**, 311.

[20] Holmgren, E., *Anat. Anz.*, 1907, **31**, 609; *Arch. f. mikr. Anat.*, 1907-08, **71**, 165; *Ibid.*, 1910, **75**, 240; *Anat. Anz.*, 1913, **44**, 225; *Nevraxe*, 1913, **14-15**, 277.

[21] Albrecht, E., *Verhandl. d. deutsch, path. Gesellsch.*, 1903, **6**, 63.

[22] Bell, E. T., *Anat. Rec.*, 1910, **4**, 199; *Internat. Monatschr. f. Anat. u. Physiol.*, 1911, **28**, 297; *J. Path. and Bact.*, 1912-13, **17**, 147.

[23] Carey, E. J., unpublished observations.

[24] Carey, E. J., unpublished observations.

agranular muscle fibers. The nerve endings are usually retracted in the dark, coarsely granular fiber and expanded in the light, agranular fiber, as revealed by gold impregnation of teased whole muscle fibers. The atrophy of disuse following tenotomy of the gastrocnemius muscle with nerve supply intact, is accompanied, during the first month, by the progressive loss of the narrow and dark, coarsely granular muscle fiber, and by a depletion of its innervation. The dark, granular muscle fiber is determined by the reciprocal interaction of the normally intact and attached muscle fiber with its normally functioning innervation. During the process of atrophy of disuse after tenotomy, small and giant fusiform neurosomes are discharged from the altered nerve endings. It is assumed that these giant fusiform neurosomes are the product of a retardation in the rate of the discharge, diffusion, and disappearance by hydrolysis, following tenotomy and disuse atrophy of the innervated gastrocnemius muscle. There appears to be a parallelism between the atrophy by disuse of tenotomized muscle and the loss of the normal discharge of neurosomes from the altered and progressively depleted innervation of the muscle. One factor in the atrophy of disuse of muscle appeared, therefore, to be the substantial loss of the discharge of neurosomes into muscle as well as the quantitative decrease of the myoplasm. The giant fusiform neurosomes that appear during the early period following tenotomy disappear when the living muscle *in situ* is adequately restretched prior to excision and gold impregnation.

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Ornithosis in Sea-Shore Birds.*

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P. [illegible]ke infections have been reported [illegible] a [illegible]ge variety of wild and domesticat[illegible] birds including parrots, finches, pigeo[illegible] chickens and turkeys.[1] Because of the w[illegible]e prevalence of psittacosis-like infection among many species of wild and domesticated birds, Meyer proposed that the more general term "ornithosis" be employed in referring to nonpsittacine infections. It has not yet been found in partridges, quail or wild ducks.[2] Among the sea birds, the Fulmar petrel has been incriminated as the source of an epidemic of psittacosis in human inhabitants of the Faroe Islands.[3] Many of the avian species in which this disease is present ordinarily do not manifest clinical symptoms of the disease but carry the virus in "silent" fashion. This dormant infection of birds appears to be provoked to activity by conditions associated with crowding, malnourishment, or other effects of commercial aviary mismanagement. The disease might also be regarded as a natural population controlling factor, remaining inapparent until overpopulation and undernourishment permit the infection to become activated and aggravated in virulence.

Psittacosis (ornithosis) is an important public health problem; so it is important that the natural or potential reservoirs of the disease be known. A study of the incidence of ornithotic infections among sea shore birds

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2 Eddie, B., and Francis, T., Jr., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 291.

3 Rasmussen, R. F., *Zentralbl. f. Bakt.*, I Abt., Orig., 1938, **143**, 89.