dilatation of the bile capillaries but also apposition of phosphatase around them which increases with the duration of the experiment. The changes in the distribution of alkaline phosphatase in histochemical preparations favor the assumption that the increase of serum phosphatase in liver damage is due to the inability of the liver cells to excrete the enzyme rather than to increased production in the liver itself.

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Morphologic Effects of DDT on Nerve Endings, Neurosomes, and Fiber Types in Voluntary Muscles.*

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The effect of DDT (2, 2 bis(p-chlorophenyl)-1,1,1-trichloroethane) on the voluntary neuromuscular apparatus in the living, intact animal is unknown. Lewis and Richards[1] state that DDT does not affect the growth of various cells in 7- to 8-day chick embryos and in a 1-day rat in hanging drop cultures. They are unable to explain the apparent paradox of the toxicity of DDT to intact, living animals and its non-toxicity on their isolated cells in hanging drop cultures. Yeager and Munson[2] have presented physiological evidence for a possible site of action of DDT in bringing about repetitive discharges of nerve impulses into the muscles of the roach, *Periplaneta americana,* which indicates that the site (or sites) referred to is that region of a nerve which lies between the origin of its fibers in the ventral nerve cord and the termination of its fibers in the leg, exclusive of the origin and the endings, that is, the myoneural junctions. Their results suggest that DDT can act more readily on motor than on sensory nerves and, further, that DDT stimulates motor nerves somewhere along their course between the cells of origin in the ventral nerve cord and the nerve endings in muscle.

The peripheral effects of DDT on the living, nerve-intact muscle, nevertheless, are visibly evident in chameleons and rats, in the persistent, involuntary, clonic contractions and occasional epileptoid convulsions and violent tremors of the entire voluntary musculature until complete flaccid or spastic paralysis and death occur. The purpose of this paper, therefore, is to demonstrate the striking morphologic changes observed in the voluntary neuromuscular apparatus of chameleons and rats during the transmission of a quantitative increase of neurogenic substances into the myoplasm of some muscle fibers affected by DDT.

Methods. A quick-killing emulsion[1] was prepared which contained 1% DDT, 8% olive oil, 1% gum arabic, and 90% Locke's solution; 2 cc of this emulsion was injected intraperitoneally into 48 white rats *(Mus norvegicus)*, average weight 250 g, and 0.1 cc into 100 summer pseudo-chameleons *(Anolis caroliniensis)*, average weight 3 to 8 g. Eight rats were selected at 12-hour intervals after the injection of DDT for microscopic study of the gastrocnemius muscle, sciatic nerve, and spinal cord. Various histologic technics were employed for the study of Nissl substance, myelin sheath, axis cylinders, motor end plates, and liposomes in muscle. Ten chameleons were selected also, but at 2-hour

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc., and the Baruch Committee on Physical Medicine.

1 Lewis, W. H., and Richards, A. G., Jr., *Science*, 1945, **102**, 330.

2 Yeager, J. F., and Munson, S. C., *Science*, 1945, **102**, 305.

intervals after the injection of DDT because of the more rapid action of DDT in the chameleon than in the rat. The same microtechnics were employed to study the muscles, peripheral nerves, and spinal cords of the chameleons as in the rat.

The lower extremities in 10 chameleons were quickly skinned and then immersed vertically in Locke's solution, 50°C, for 10 seconds. The muscles of the lower extremities, immediately upon immersion, manifested heat rigor. After immersion, the muscles were quickly excised and prepared for microscopic study. The bloodless muscles of the lower extremities from 10 DDT rats were excised after the muscles were perfused, through the arch of the aorta, with physiological salt solution until clear fluid poured out of an incision in the right auricle of the heart. The muscles were dehydrated *in vacuo* and fed to 10 rats. Seven of the 10 rats manifested DDT toxicity within 3 days. Two hundred fifty mg of dehydrated and triturated muscle, previously perfused, from rats killed with DDT, were suspended in 10 cc of olive oil. Five-tenths of one cc of this suspension were injected intraperitoneally into each of 20 chameleons. Fourteen manifested DDT toxicity within 48 hours and 6 were unaffected. The muscles, peripheral nerves, and spinal cord from 10 normal white rats and 10 chameleons quickly killed by ether were excised, subjected to the microscopic methods of preparation enumerated above, and used as controls. The microscopic observations reported in this paper will be confined dominantly to those demonstrated by the gold technic previously described.[3]

Tests were made *in vitro* of the chemical reactions of 1% gold chloride with 1% acetylcholine chloride, and of 1% gold chloride with 1% choline chloride. Precipitates of acetylcholine aurichloride and choline aurichloride, respectively, were formed.[4] When this reaction occurred in capillary glass tubes with an inside diameter of 100 μ, evanescent cross striations of periodic precipitation formed, which subsequently disappeared in a uniform diffusion of granules. One percent osmic acid formed a precipitate both with 1% acetylcholine chloride and 1% choline chloride. One percent gold chloride also produced a precipitate with 1% alcoholic solution in each of the following: oleic, palmitic, and stearic acids. There was likewise an interaction between gold chloride and lecithin, and a very strong interaction in the reduction of gold by 1% ascorbic acid and by 1% thiamine hydrochloride.

Results. After the intraperitoneal injection of DDT in the rat, death occurred within 12 to 72 hours, and in the chameleon within 6 to 24 hours. Forty-one of the 50 rats had definite chromodacryorrhea due to overstimulation of the harderian glands which secreted the red-brown porphyrin pigment. This was comparable to the toxic effects of acetylcholine. All the rats had intense salivation, involuntary passage of feces and urine, and clonic, involuntary contractions and tremors of the voluntary muscle, which were the usual physical manifestations of toxicity due to DDT in the rat. In the chameleon the pigmentation of the skin usually changed from green to dark brown, and there were persistent, intermittent contractions of the voluntary muscles. When the chameleons in the terminal stages of DDT toxicity were skinned, there was a continuation of intermittent and incoordinate muscle action that resembled both fibrillation and fasciculation of denervated muscle. This was likewise observed in isolated muscles for 10-20 minutes after excision at 22°C.

The salient microscopic morphologic changes in 0.5% to 15% of the motor innervations in 100 rat and 200 chameleon gastrocnemius muscles after toxicity with DDT (Fig. 2, 3, and 6), in contrast to the normal control muscles (Fig. 1, 4, and 5) in the teased whole muscle fibers and cross sections, were the following: (1) progressive depletion of some epilemmal axons of auriphilic substance and demyelinization of some peripheral nerves; (2) centrifugal discharge from the motor end plate of these auriphilic neurosomes into the myoplasm either as elongated streamers, serial beads, or large elongated masses; (3) the appearance of this auriphilic

[3] Carey, E. J., *Anat. Rec.*, 1941, **81**, 393; *Am. J. Path.*, 1942, **18**, 237.

[4] Loach, J. V., *J. Physiol.*, 1934, **82**, 118.

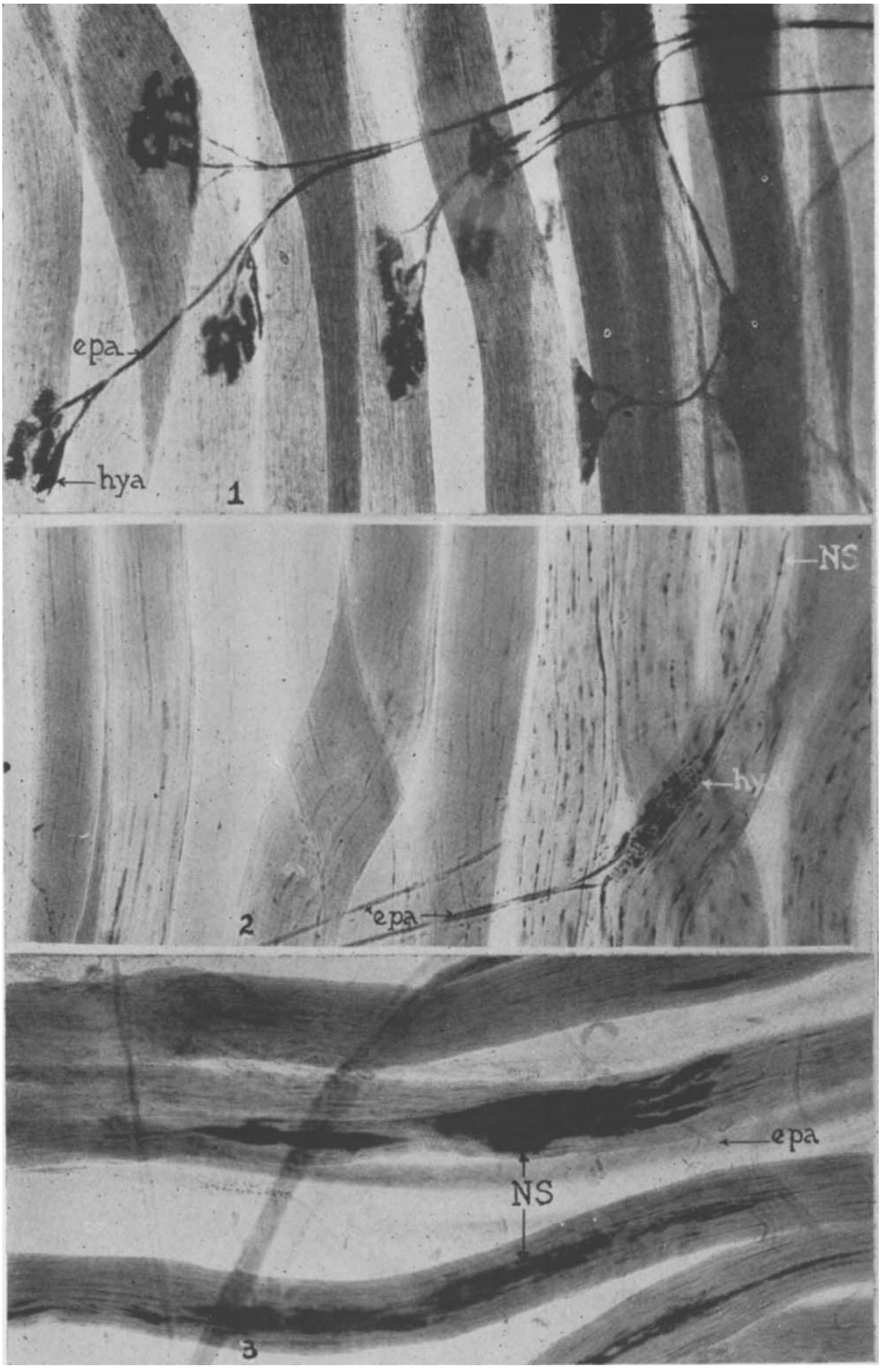

FIG. 1 to 3.

Photomicrographs × 200. Normal innervation, Fig. 1, discharge of massive neurosomes from end plates by DDT, Fig. 2 and 3. There is progressive depletion of auriphilic substances in the epilemmal axons, Fig. 2 and 3, contemporaneous with the appearance of the auriphilic neurosomes in the myoplasm. Legend: epa, epilemmal axons; hya, hypolemmal axons; NS, neurosomes. Gold chloride; teased whole gastrocnemius muscle fibers; chameleon.

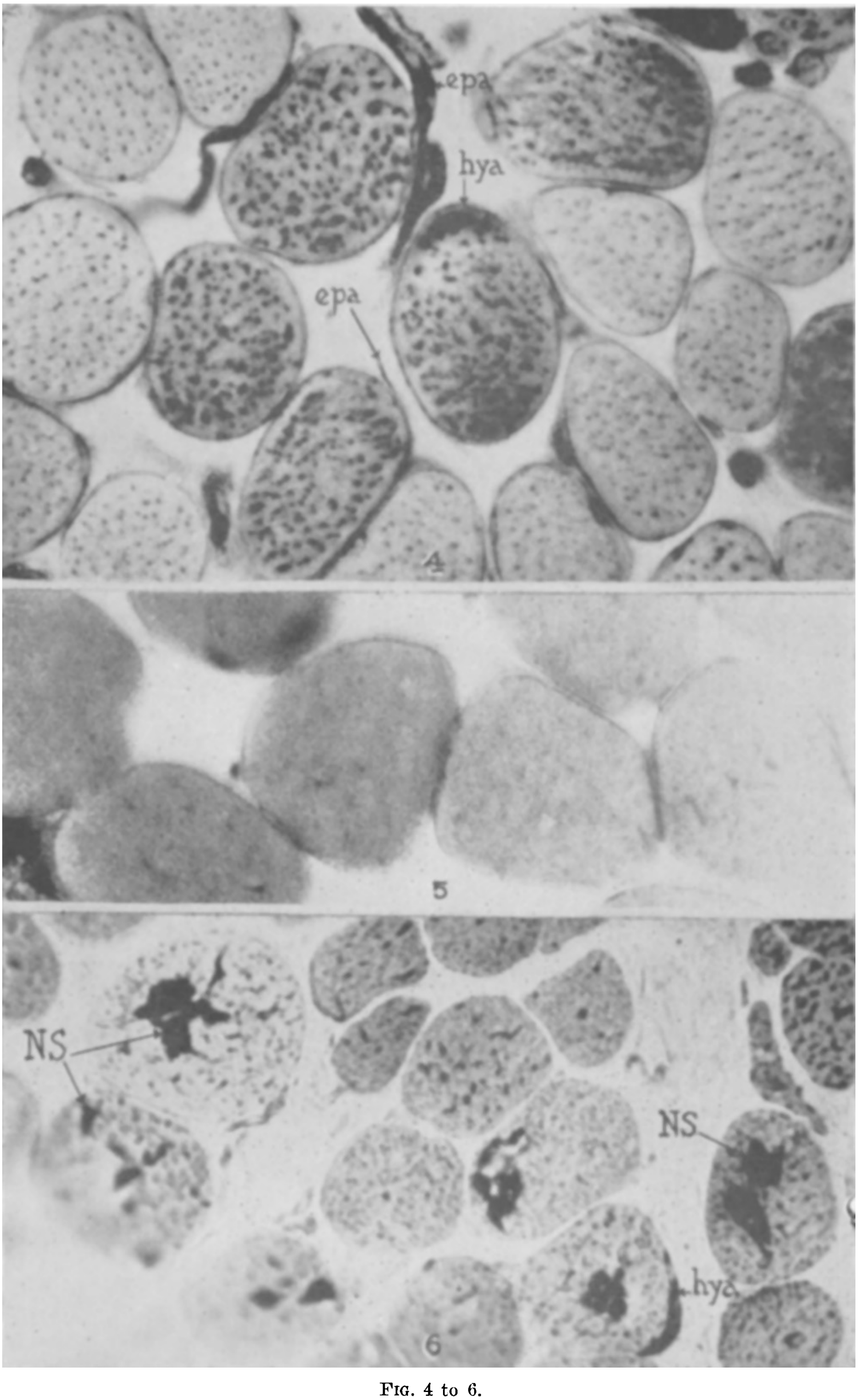

FIG. 4 to 6.

Photomicrographs × 500. Normal coarsely and finely granular muscle fibers, Fig. 4; and agranular fibers, Fig. 5; and agglutination of neurosomes, NS, after DDT, Fig. 6. Legend: epa, epilemmal axons; hya, hypolemmal axons. Gold chloride; cross sections, gastrocnemius muscle, chameleon.

neurogenic substance in the myoplasm of the muscle fiber by jet-pump action from, and in anatomical continuity with, the motor end plate (Fig. 2 and 3); (4) the subsarcolemmal position of the agglutinated islands of augmented auriphilic substance, seen in whole teased muscle fibers (Fig. 3) and in the cross sections (Fig. 6); (5) an acute atrophy occurring in some muscle fibers affected by DDT (Fig. 6); (6) disappearance of the motor end plates in 10% to 30% of the biopsied gastrocnemius muscles, from 10 chameleons and 10 rats, during the terminal stage of DDT paralysis.

There was an acute atrophy of the muscle fibers in some places. In some muscle fibers dispersion of the granules was lost completely. The granules were agglutinated in large masses in localized spots in some muscle fibers; and this was comparable to the loss of granules in some parts and aggregation in other parts of the specific granular muscle fiber observed 10-18 days after denervation[5] and after 12 days of total starvation,[6] except water, in the rat. In these denervated gastrocnemius muscles the fine neurosomic granules of Kühne disappeared around the degenerated nerve endings about the 72nd hour after section of the sciatic nerve. There were, however, centrifugal discharges of large, coarse granules into the muscle from the greatly altered nerve terminals, until the distal stump of nerve was depleted of degenerated axonic and myelin substances. This final depletion stage of the distal stump of the sectioned nerve varied in individual rats from 10 to 18 days. The specific structure and dispersal of granules, therefore, in the dark muscle fibers appeared to depend upon the intact nerve endings and normal function of the innervation of muscle.

In the normal muscle fiber of the chameleon there was variation in the distribution of the granular and agranular muscle fibers. The agranular muscle fibers were increased in number by heat rigor of short duration produced by submerging the skinned chameleon in Locke's solution for 10 seconds at 50°C. The agranular fibers produced by this short duration of heat rigor were comparable to the normal agranular fibers (Fig. 5). In heat rigor 90% to 96% of the muscle fibers were agranular, whereas in the normal only 40% to 75% were agranular, 20% to 58% finely granular, and only 2% to 5% coarsely granular. This differential count was based on the observations of 2000 fibers in each of 20 gastrocnemius muscles from 10 chameleons: a total of 40,000 fibers. Although the muscle fibers with greatest diameter were usually pale and agranular, this was not an invariable finding. In the winter animal there was a number of pale fibers with very fine granules, or agranular, that possessed small fiber diameters. Some other medium-sized muscle fibers of the dark type were packed with coarse granules. The normal granules of the muscle fibers varied in diameter from 0.2 to 10 μ.

Some of the auriphilic granules formed elongated bipolar streamers in relation to the muscle nuclei. The granules in the muscle fiber, therefore, were dual in origin: (1) neurosomes discharged from their motor nerve endings and diffused in the myoplasm; and (2) granules discharged from the 2 terminal poles of each of the multiple elongated nuclei in the protoplasm of the muscle. In some places the granules related to the muscle nuclei and the neurosomes continuous with the nerve endings appeared to intermingle and coalesce to form one elongated granular mass. There were many nuclei located in the center of the granular fibers whereas most of the nuclei were located under the sarcolemma in the agranular muscle fibers.

The abnormal auriphilic masses of neurogenic substance produced by DDT were sometimes as long as 450 μ and had in some places (Fig. 3 and 6) a diameter of 50 μ. The normal granules and the abnormal masses of auriphilic neurogenic substance were inconstant in their refractive properties, size, shape, number, and capacity to react to gold, silver, methylene blue, osmic acid, sudan III and IV, sudan black, and alkaline scarlet red. The part of a muscle fiber depleted of its gold staining substance was definitely

[5] Carey, E. J., Massopust, L. C., Haushalter, E., Sweeney, J., Saribalis, C., and Raggio, J., *Am. J. Path.*, in press.

[6] Carey, E. J., *Anat. Rec.*, 1942, **82**, 403.

agranular when there was an aggregation of this substance produced by DDT in another part of the same fiber. The massive aggregation of auriphilic substance in the myoplasm of the voluntary muscle fiber was in anatomic continuity in many instances with the auriphilic substance of the hypolemmal and epilemmal axons undergoing depletion by the action of DDT. That at least some of the auriphilic masses were neurogenic in origin is proved by the following microscopic evidence: (1) there is frequently a continuous anatomic relationship between this auriphilic substance in the muscle and the nerve ending; (2) there is a similarity of reaction of this substance in nerve and muscle with gold; (3) there is an excessive and massive accumulation of this auriphilic substance in muscle parallel with the exhaustion of the nerve supply of its auriphilic substance.

Discussion. The mechanism of production of the granular and agranular muscle fibers is still unknown. This fact has been pointed out in the relatively recent excellent reviews and observations by Cobb,[7] Needham,[8] Hines,[9] Hinsey,[10] Denny-Brown,[11] and Tower.[12] The histologist uses various synonyms dependent upon observations made on fresh muscle or on those made after chemical alteration by fixatives and staining reactions for the "granular and agranular" muscle fibers, respectively, as follows: "dark and light or pale," "opaque and clear," "plasmic and aplasmic," "red and white," muscle fibers. Grützner[13] stated that all granular muscle fibers are red muscle fibers and that the agranular are white muscle fibers. Starling[14] claimed that all striated muscles of higher vertebrates are microscopically mixed muscles and contain both red and white types of muscle fibers. It was demonstrated by Knoll[15] that the granules in the small muscle fiber can be stained with osmic acid and gold. Kölliker,[16] Albrecht,[17] and Bell,[18] identified these granules as lipoid in nature, basing their identification on reactions with osmic acid and fat stains such as sudan III and scarlet red. However, we present evidence in this paper that choline and acetylcholine likewise react strongly with both osmic acid and gold chloride.

Krause[19] stated that the small granular muscle fiber was a young form that eventually grew into the adult large agranular fiber. Bonhöffer[20] studied the same muscle at different ages and found the distribution of the granular and agranular fibers was about the same at different periods in development. Schaffer[21] reported that the arrangement of the coarse myofibrils was without order in the granular fiber whereas there was more regularity in the distribution of the fine myofibrils in the agranular fiber into so-called areas of Cohnheim, in cross sections, or columns of Kölliker, in longitudinal sections. The so-called sarcoplasm was greater in red granular fibers than in white agranular muscle fibers according to Schaffer.

Experimental evidence is presented here which supports the assumption that there is a periodic shift in the location of the muscle nuclei in relation with the phase of the cycle of metabolism stopped by gold impregnation.

7 Cobb, S., *Physiol. Rev.*, 1925, **5**, 518.

8 Needham, D. M., *Physiol. Rev.*, 1926, **6**, 1.

9 Hines, M., *Quart. Rev. Biol.*, 1927, **2**, 149.

10 Hinsey, J. C., *J. Comp. Neurol.*, 1927, **44**, 87; *Physiol. Rev.*, 1934, **14**, 514.

11 Denny-Brown, D. E., *Roy. Soc. London, Proc.*, Series B, 1929, **104**, 371.

12 Tower, S. S., *Physiol. Rev.*, 1939, **19**, 1.

13 Grützner, P., *Pflüger's Arch.*, 1887, **41**, 256.

14 Starling E. H., *Principles of Human Physiology*, 5th ed., edited and revised by C. Lovatt Evans, Lea & Febiger, Phil., 1930, p. 139.

15 Knoll, P., *Z. f. Heilk.*, Berlin, 1880-81, **1**, S. 255; *Denkschr. d. k. Akad. d. Wissensch. Wien. Math. Naturw. Kl.*, Wien, 1891, **58**.

16 Kölliker, A., *Z. f. Wissensch. Zool.*, Leipzig, 1857, **8**, 311; *ibid.*, 1888, **47**, S. 689.

17 Albrecht, E., *Deutsche. path. Gesellsch.*, 1902, **5**, S. 7; *ibid.*, 1903, **6**, S. 63; *Ergeb. der allg. Path. u. Path. Anat.*, 1907, **11**, Abth. 2, S. 1166.

18 Bell, E. T., *Anat. Rec.*, 1910, **4**, 199; *Internat. Monatsch. f. Anat. u. Physiol.*, 1911, **28**, S. 297; *J. Path. and Bact.*, 1912, **17**, 147.

19 Krause, W., *Die Motorschen Endplatten der quergestreiften Muskelfasern*, Hannover, Hahn, 1869, pp. 192, and one plate.

20 Bonhöffer, K., *Pflüger's Arch.*, 1890, **47**, 125.

21 Schaffer, J., *Sitzungsb. d. k. Akad. d. Wissensch. Math.-Naturw. Kl.*, Wien, 1893, **102**, Abth. 3, S. 7.

It is suggested that the locations of the muscle nuclei are related to the mechanical effects of the chemical reactions produced by the discharge, hydrolysis, and disappearance of the granular neurosomes projected into the myoplasm from the nerve endings. It is likewise suggested that the fiber types in muscle appeared not to be fixed histologic structures but that they readily are transformed one into the other, dependent upon the phase of the metabolic cycle of nerve and muscle in which the tissue ceased activity by the impregnation with gold. The periodic changes in the histochemistry of muscle appeared to determine the granular and the agranular types of muscle fibers independently of the gross color of the muscle. The observations reported in the chameleon in this paper are made upon the grossly pale reptilian muscle, and the granular fibers have no relation to the so-called "red" muscle.

The same axis cylinder may divide and end in 2 motor end plates, one retracted in the granular muscle fiber and the other expanded in the agranular muscle fiber. This is suggestive evidence that the same axis-cylinder of a nerve, upon division, may terminate in muscle fibers fixed in different phases of either the discharge or hydrolysis of neurosomes in the metabolic cycle of nerve and muscle. The greater number of granular fibers found in the slower than in the faster reacting muscle is assumed to be related to the differential speed of metabolism and response to the chemical irritation produced by gold impregnation. If this assumption is true, then a greater number of granular fibers would be found in the slower than in the faster reacting muscles. These granular fibers, therefore, are not related to hemoglobin pigmentation and the adjective "red" should be discarded.

Denny-Brown[11] cut the ventral roots of the sciatic nerve in the cat. Two weeks later he found that the distribution and depth of staining of dark and light fibers in the gastrocnemius and soleus had remained the same on the operated side as on the control side. We have duplicated this experiment in 50 rats[5] and 10 cats. The definitive dark fibers disappear in from 10 to 18 days in the rat, and from 21 to 36 days in the cat. There is no way, based upon the use of either fat stains of alkaline sudan III, scarlet red, osmic acid or metallic impregnation with gold or silver, of clearly differentiating from one another acetylcholine, choline, lecithin, and other products of the breakdown of complex lipoids. Seventy-two hours after nerve section in the rat, acetylcholine is not identified by Dale, Feldberg, and Vogt,[22] yet there is a periodic discharge of some complex lipoids, in the form of very coarse granules, from the degenerated end plate, and these granules react to osmic acid, alkaline sudan III, and to gold chloride. This centrifugal discharge of the degenerated axonic and myelin material of the distal stump of the cut sciatic nerve into the denervated muscle continues until complete substantial exhaustion occurs. It is because of this fact, and species variation in degeneration of nerves, that Denny-Brown[11] did not observe any great change in the disappearance of the muscle granules in the narrow, dark fibers 14 days after nerve section in the cat. The dark, granular muscle fiber disappears when a sufficient length of time has elapsed after nerve section as well as after starvation. Denervation[5] results in a material, lipoidal exhaustion of the distal stump of the sciatic nerve, whereas starvation[6] produces either a partial or complete block in the substantial transmission of neurogenic substances in some fibers of the muscle. This is evident by the great enlargement of some of the nerve axons extrinsic to the sarcolemma[6] and the retraction of the hypolemmal axons and disappearance of the granules of Kühne.

The effect of DDT on the neuromuscular apparatus results in an augmented discharge and aggregation of neurosomes in the myoplasm. DDT produces dissociation of the neurogenic and myogenic substances in the voluntary muscle fiber. DDT likewise finally produces a chemical denervation of some muscle fibers microscopically detected by the disappearance of some of the motor end plates. DDT also causes demyelinization of some

[22] Dale, H. H., Feldberg, W., and Vogt, M., *J. Physiol.*, 1936, **86**, 353.

of the intramuscular medullated nerves.

The augmentation and aggregation of neurogenic substances in the voluntary muscle fibers affected by DDT parallels the abnormal response and subsequent paralysis through axonic exhaustion by chemical denervation of some muscle fibers. It is suggested that the normal dark and granular muscle fiber be designated as the neurosomic muscle fiber and that the light and agranular fiber be called the aneurosomic muscle fiber. This terminology is based on the hypothesis that there is a periodic discharge and disappearance of the neurogenic granules in the fibrillar protoplasm of the same voluntary muscle fiber observed at different periods of time, and the hypothesis itself is supported by the suggestive experimental evidence of the effects of DDT.

The observations reported in this paper are, in general, consistent with the views expressed, over one hundred years ago, by Doyère.[23] He observed the junction between the living nerve and muscle in the microscopic Tardigrade (Spallanzani) also called the "water bear." Its body is a transparent muscular bag. Doyère stated, page 346, that "the relation of the terminal nerve filaments with the muscles cannot be distinguished," and in Fig. 4, plate 17, he illustrates the junction of the granular nerve fiber and the granular muscle fiber as morphologically continuous.

Summary. The limited experimental evidence presented in this paper tends to support the statement that DDT increases the discharge of auriphilic neurogenic substances from some motor end plates into the myoplasm of some of the voluntary muscle fibers. These neurogenic substances form massive aggregates of neurosomes in parts of some voluntary muscle fibers. This results in a partial or complete dissociation of the neurogenic from the myogenic substances in the muscle fiber. Auriphilic granules are likewise found at the 2 poles of the elongated nuclei in the dark muscle fibers. The supporting evidence that some of the auriphilic granules and masses found after DDT toxicity are neurogenic in origin follows: (1) anatomical continuity of the auriphilic bodies in muscle with the auriphilic hypolemmal axons of the motor end plates; (2) similarity of staining reaction of the masses in muscle and axons of nerve endings with gold impregnation; (3) massive aggregation of auriphilic bodies in some muscle fibers contemporaneous with the centrifugal depletion of the related nerve axons and endings of their auriphilic substances. Presumptive evidence is presented that the normal fiber types in voluntary muscle are dependent, in part, upon the periodic alternation of the discharge and disappearance, by chemical action, of the neurogenic granules in the same muscle fiber at different time periods. The limited evidence also indicates that DDT poisoning may be produced in normal rats by feeding, and in chameleons by the intraperitoneal injection of the previously perfused, dehydrated, and emulsified muscles of rats manifesting toxicity with DDT.

23 Doyère, M., *Annales d. Sciences Naturelles*, second series, 1840, **14**, 269.

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Diffusion of Sulfonamides and Penicillin into Fibrin.*

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Until the advent of penicillin, subacute bacterial endocarditis largely resisted all forms of therapy. The persistence of the disease is due to the survival of the bacteria in the valve locus. The organisms in the vegetations are separated from the blood stream by a layer of fibrin and it has been

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