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A Mammalian Nerve—Muscle Preparation Suitable for Single-Fiber Experiments

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Studies on impulse transmission from nerve to muscle were facilitated when techniques were evolved for conducting experiments on single-fiber preparations. In the main, cold-blooded animals have been used (frog, *1, 2, 3,* and lizard, *4*). The isolation of a single mammalian muscle fiber with an intact blood supply and unbroken nervous connections has proved difficult in the past (*5, 6*). Investigations in this department requiring such biological material have led to the discovery of a suitable preparation in the M. serratus anterior of the guinea pig.

Exposure of the M. serratus anterior, and its motor nerve, is accomplished by division of the overlying M. pectoralis major and the M. rhomboidei. The M. serratus anterior has digitations consisting of parallel muscle fibers interconnected by, and enveloped in, a delicate transparent membrane. The fibers are unobscured by other major connective tissue. At its edges

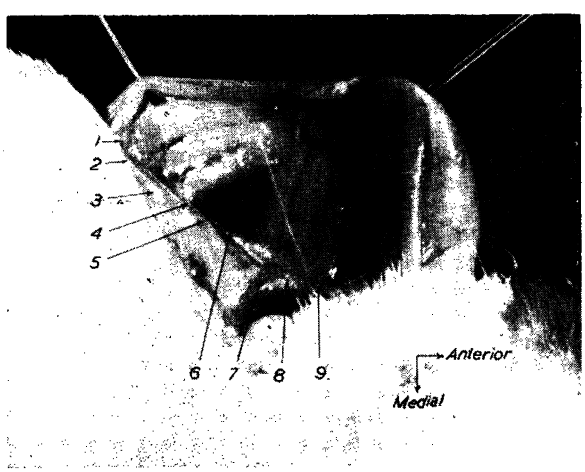

FIG. 1.

each digitation has a depth of only 1 or 2 muscle fibers. Upon laterad deflection of the scapula, the trunk and branches of the N. thoracalis longus, which furnish the motor nerve supply to the M. serratus anterior, are easily seen (Fig. 1). The end plates, or myoneural junction tissue, of the unstained living cells are readily distinguished under the microscope. A photomicrograph of such a preparation (Fig. 2)

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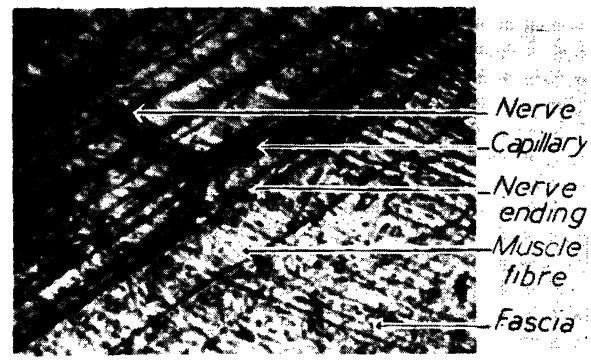

FIG. 2.

shows junction tissue between a muscle fiber and a nerve twig. The vascular supply of the muscle fibers is apparently not disturbed despite the abnormal position of the muscle. If the tissues are adequately irrigated with warm physiological saline or mineral oil the muscle fibers respond to electrical stimulation of the motor nerve for several hours after exposure. The fibers and end plates can be touched and pierced with micropipettes and microelectrodes.

The tendons of the M. serratus anterior are too short to permit easy dissection of the muscle away from its insertions. There is no difficulty, however, in removing the muscle, together with the bones upon which it is inserted, to provide an avascular preparation, which is of advantage at times.

This mammalian preparation may prove useful in various branches of physiology and pharmacology.

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Fluorescence and Photoinactivation of Snake Poisons

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It has been shown by Fonseca Ribeiro and Guimarães (*1*) that potassium chlorophyllinate becomes active for the inactivation of the poison of *Crotalus terrificus terrificus,* either through aging or through light exposition. The mechanism of this phenomenon has not been satisfactorily explained.

In a recent study Ferri (*2*) was able to demonstrate that the photoinactivation of indolacetic acid (phytohormone) by riboflavin discovered by Galston (*3*) should be explained by a mechanism in which riboflavin did not act specifically, since the same inacti-

[1] The authors wish to express their appreciation to Fonseca Ribeiro, Mário G. Ferri, and Annibal A. Pereira.

vation could be brought about by many different substances. Although chemically unrelated, all these compounds had in common the property of fluorescence.

This fact led us to investigate whether the chlorophyll inactivation of snake poison could be attributed to the fluorescent substances that might possibly have been present, even if in very small quantities, in the K-chlorophyllinate solutions. The poison used in the experiments was that obtained from animals of the genus *Bothrops*.

Riboflavin, quinine sulfate (colorless, but with a visible fluorescence), γ-eosin, fluorescein, and K-chlorophyllinate Baker were employed in aqueous solutions of different concentrations as possible photosensitizers. The tests for the toxicity of the preparations were made in pigeons.

The experiments were performed in the following way: 2, 10, or 20 MLD (minimal lethal doses for pigeons) were added to solutions of fluorescent substances. These solutions were then divided in 2 aliquots (containing respectively 1, 5, and 10 MLD), one of which was kept in the dark and the other exposed to direct sunlight for 90 min.

Two ml of each solution was then injected intravenously in pigeons. The action of *Bothrops* poison is detectable within 20 min, but the animals were kept under observation for 4 days.

The results of the experiment are presented in Table 1.

TABLE 1

A. Aqueous solution of riboflavin, 0.01%

Pigeon No.	Wt (g)	Vol injected intravenously (ml)	Observations
1	240	1	Normal behavior
2	260	1	" "

B. 1 ml aqueous solution of riboflavin, 0.01% added of *Bothrops* poison; kept in dark for 90 min

Pigeon No.	Wt (g)	Poison injected intravenously (MLD)	Observations
1	250	1	Normal behavior
2	240	5	Died in 1 min, 30 sec
3	310	10	" " 45 sec

C. 1 ml aqueous solution of riboflavin, 0.01%, added of *Bothrops* poison; exposed to direct sunlight for 90 min

Pigeon No.	Wt (g)	Poison injected intravenously (MLD)	Observations
1	250	1	Normal behavior
2	240	5	" "
3	230	10	" "
4	310	30	" "

TABLE 2

A. Aqueous solution of quinine sulfate, 0.1%

Pigeon No.	Wt (g)	Vol injected intravenously (ml)	Observations
1	240	1	Normal behavior
2	270	1	" "

B. 1 ml aqueous solution of quinine sulfate, 0.1% added of *Bothrops* poison; kept in dark for 90 min

Pigeon No.	Wt (g)	Poison injected intravenously (MLD)	Observations
1	300	1	Died in 8 min, 30 sec
2	310	5	" " 2 " 2 "
3	270	10	" " 1 " 5 "

C. 1 ml aqueous solution of quinine sulfate, 0.1% added of *Bothrops* poison; exposed to direct sunlight for 90 min

Pigeon No.	Wt (g)	Poison injected intravenously (MLD)	Observations
1	280	1	Normal behavior
2	230	5	" "
3	250	10	" "

It may be seen from the data presented that riboflavin alone, as expected, had no effect on the animal behavior. All pigeons injected with the mixture of riboflavin and poison which was kept in the dark died within a few seconds, with only one exception.

All pigeons treated with the light-exposed mixtures remained alive and did not show any poisoning symptoms. It may be concluded from these data that riboflavin is capable of counteracting the toxicity of snake poison in the presence of light but not in the dark. The exception referred to could be due to some accidental exposure to light during the manipulations.

This experiment was repeated with the same results. Analogous experiments in which riboflavin was substituted for one of the other fluorescent compounds mentioned above gave essentially the same results (Table 2).

It seems to us that these data leave no doubt about the fact that the inhibition of the poisoning effect is by no means peculiar to chlorophyll but is a property common to many fluorescent substances. This process thus seems to be analogous in nature to the *in vitro* photoinactivation of indolacetic acid sensitized by fluorescent substances (*2*).

These results led us to investigate whether other properties of the snake poison were altered by light in presence of the fluorescent substances. We shall mention now only the fact that the power of coagulating the blood plasma was almost completely maintained even under circumstances in which the toxicity

was completely removed. Work in this area is under way, and we believe that many important practical applications may arise from the findings.

Other questions being investigated by us are: Is the antigenic property of the poison unaltered by exposure to light in the presence of the fluorescent substance? Are the fluorescent compounds effective in bringing about the inactivation by light of such other toxins as tetanal, diphtherial, staphylococcus, and gangrenous? Should the second question be answered affirmatively, it would then be possible to find a much easier way of preparing vaccines against these toxins and related diseases.

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Nonequivalence of Methyl and Carboxyl Groups in Photometabolism of Acetate by *Rhodospirillum rubrum*[1]

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It has been shown that the distribution of labeled carbon in carbonate and cell material produced during the dark aerobic dissimilation of C^{14}-labeled acetate by the photosynthetic bacterium *Rhodospirillum rubrum* is the same whether the acetate is labeled initially in the methyl or in the carboxyl group.[4] Hence, it is of interest to report that this equivalence of acetate carbons is not evidenced when labeled acetate is dissimilated photochemically by *R. rubrum*. Typical results are shown in Table 1. To facilitate direct comparison of different experiments, the data have been normalized to the same initial conditions, and amount of acetate metabolized. Experimental uncertainty in any of the values shown is less than 10%.

It will be noted that when methyl-labeled acetate is dissimilated photochemically the distribution in the end products of metabolism differs radically from that observed under identical conditions using carboxyl-labeled acetate. Thus, when 30 μM of acetate are photometabolized anaerobically, the methyl carbon finds its way practically entirely into insoluble cell material, whereas a large fraction of the carboxyl carbon appears as carbonate. Dark aerobic oxidation of the same quantity of methyl-labeled acetate results in the usual accumulation of labeled carbon in carbonate. For comparison of labeled carbonate production in light and dark metabolism it should be noted that observed yields of carbonate per mol acetate disappearing are 0.20 to 0.25 and 0.6 to 0.8 mols, in light and dark, respectively.

The labeled carbon content of the soluble cell material is of the same order of magnitude regardless of the experimental conditions used (Table 1). Extensive analysis of this fraction is not warranted because of the long duration of the dissimilation (~1 hr). However, it may be remarked that ~30% of the activity in this fraction can be identified by paper chromatography as tricarboxylic acid cycle intermediates (e.g., citrate, ketoglutarate, succinate, etc.). No increase in incorporation of labeled acetate carbon in this fraction is noted when carrier amounts of tricarboxylic acid intermediates are added as trapping agents, either in light or dark metabolism. Nor are any changes in distribution of acetate carbon observed when unlabeled substrates that evolve large amounts of CO_2 (malate, succinate, ketoglutarate) are metabolized simultaneously with labeled acetate.

TABLE 1

DISTRIBUTION OF LABELED CARBON AFTER DISSIMILATION OF 30 μM C^{14}-ACETATE BY RESTING SUSPENSIONS OF *Rhodospirillum rubrum**

Experimental conditions	Light, gas phase, He		Dark, gas phase, air
	Methyl-labeled acetate	Carboxy-labeled acetate	Methyl-labeled acetate
Insoluble cell material (ct/min)	12,500	6,300	4,300
Soluble cell material (ct/min)	975	1,440	1,340
Carbonate (ct/min)	510	6,200	11,450

* Initial acetate, 125 μM C^{14}-acetate. All data normalized to initial C^{14} content of 50,000 ct/min. Equal densities of cell suspensions used (22 mg dry wt) in total vol 8 ml phosphate buffer, pH 6.6; 25 μM $NaHCO_3$ also present initially. 90–95% recovery of labeled acetate carbon dissimilated is obtained in cell fractions shown.

These results indicate that anaerobic photodissimilation of acetate by *R. rubrum* very probably does not involve a cyclic mechanism requiring equilibration of the 2 acetate carbons. Such a mechanism, on the other hand, is very likely operative in the dark oxidation of acetate by the same organism. Furthermore, it can be concluded in agreement with previous findings[5] that a major fraction of the acetate undergoes photoassimilation without intermediary formation of carbonate. A detailed account of these researches is in preparation.

[1] The authors gratefully acknowledge the financial support of the Charles F. Kettering Foundation.

[2] Lord Grey Memorial Fellow, University of Durham, England, 1949–50.

[3] U. S. Public Health Service Fellow, 1948–50.

[4] C. B. Van Niel and H. A. Barker, private communication; see also *Photosynthesis in Plants,* J. Franck and W. E. Loomis, Eds. Ames: Iowa State Press, 468 (1949). This observation has been confirmed in our laboratory.

[5] See Footnote 4.