Anaphylactic Reaction of Denervated Skeletal Muscle in the Guinea Pig

Abstract. *Muscle strips taken from chronically denervated hemidiaphragms of guinea pigs immunized to ovalbumin react in vitro to the administration of small amounts of this protein with a contraction similar to that of visceral muscle in the Schultz-Dale reaction. A sustained shortening is produced by histamine on denervated diaphragmatic muscle from both sensitized and nonsensitized guinea pigs.*

Owing to the enhanced chemical sensitivity and electrical instability of its surface membrane, which causes bursts of rhythmic action potentials and fibrillary contractions, voluntary muscle chronically deprived of motor innervation behaves, in some respects, like smooth muscle. This analogy, emphasized by Dale and Gaddum (*1*), led us to study the effects of antigens on denervated skeletal muscle taken from immunized animals.

Our results indicate that the functional similarities between both types of muscle cells are closer than is generally assumed and extend to their immunological responses. In fact, the denervated diaphragmatic muscle of guinea pigs sensitized to egg albumin (Ea) responds in vitro to small amounts of this protein with contractions which resemble those of visceral muscle in the Schultz-Dale reaction. Furthermore, histamine, serotonin, and bradykinin also cause contraction of the denervated diaphragm; this shows that the spread of acetylcholine(ACh)-sensitive sites, which follows the degeneration of the motor nerve (see *2*), is accompanied by the development of new receptors for the histamine, serotonin, and bradykinin.

Young guinea pigs (up to 300 g) were used in these experiments. The left phrenic nerve was sectioned in the cervical region, and a week later the animals were immunized with one subcutaneous injection of 10 mg of Ea in complete Freund adjuvant. The animals were killed 2 to 6 weeks after denervation, and the left hemidiaphragm was excised and divided in strips (5 mm wide) by cuts parallel to the direction of the muscle fibers. These strips were stored for at least 1 hour in oxygenated Krebs solution. The right innervated hemidiaphragms of all the animals and the left denervated hemidiaphragms of nonsensitized guinea pigs served as controls.

The effect of the antigen was recorded with the strips of muscle attached to an isotonic lever writing on a smoked drum. A tension of 1 gram was applied to all the preparations. The Krebs solution surrounding the muscle had the following composition (m*M*): Na^+, 142.90; K^+, 5.88; Ca^{++}, 1.26; Mg^{++}, 1.18; Cl^-, 125.22; $(HCO_3)^-$, 24.90; $(SO_4)^-$, 1.18; $(H_2PO_4)^-$, 1.18. This solution was maintained at 39°C and a mixture of 98 percent O_2 + 2 percent CO_2 was passed through it continuously.

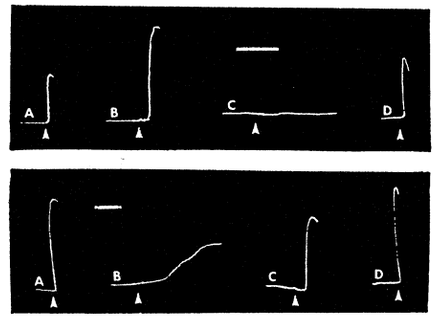

Fig. 1. Effects of acetylcholine, egg albumin, and human seroalbumin on denervated diaphragmatic muscle of a guinea pig actively sensitized to egg albumin. Upper records: *A* and *D*, acetylcholine (3 μg/ml); *B* and *C*, egg albumin (100 μg/ml). There was complete desensitization after the first dose of the antigen. Lower records: *A* and *D*, acetylcholine (3 μg/ml); *B*, human seroalbumin (100 μg/ml); *C*, egg albumin (100 μg/ml). Horizontal bar: 1 minute.

The results with denervated muscle from immunized animals can be summarized as follows.

The addition of Ea to the bath, the final concentrations being 3 to 100 μg per milliliter, caused contractions of comparable amplitude to those elicited by 1 to 3 μg of ACh per milliliter (Fig. 1). However, the time between the addition of the Ea to the bath and the onset of contraction was longer and the rate of shortening was less than those produced by ACh.

A second application of Ea produced either a smaller contraction or none at all. Generally, the greater the effect of the first dose of antigen the smaller the size of the second response. The second response to Ea occurred after a latency period longer than that following the initial administration of the antigen (Fig. 2).

In some instances, human seroalbumin (HSA) evoked a contraction, which was smaller and occurred after a longer latency than that following the first administration of Ea. When Ea was given after administration of HSA the responses were smaller. This was true even if the initial application of HSA did not produce a contraction.

Muscle contractions were also recorded after addition of small amounts of HSA to strips of denervated diaphragm taken from guinea pigs passively sensitized by the intravenous administration of rabbit antiserum to HSA.

On the other hand, neither the innervated side of the diaphragm from sensitized animals nor the denervated muscle of nonsensitized guinea pigs re-

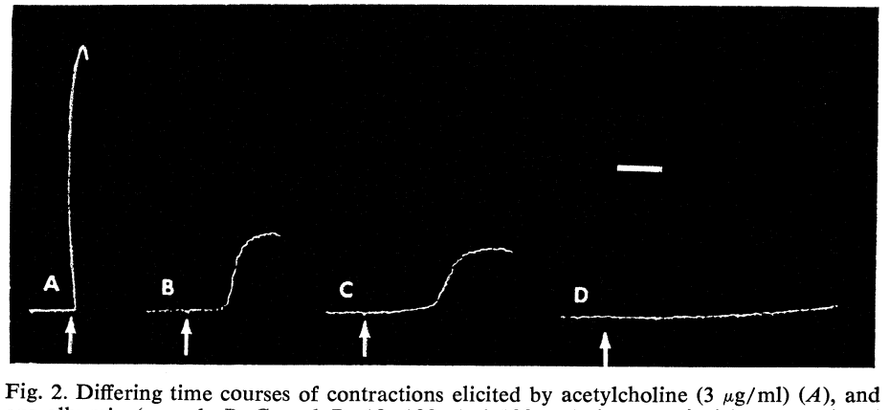

Fig. 2. Differing time courses of contractions elicited by acetylcholine (3 μg/ml) (*A*), and egg albumin (records *B*, *C*, and *D*; 10, 100, and 100 μg/ml, respectively) on a strip of denervated diaphragmatic muscle of a guinea pig sensitized to egg albumin. Horizontal bar: 1 minute.

sponded to the administration of antigen.

Histamine (0.1 to 1 μg/ml) caused a sustained shortening of the denervated diaphragmatic muscle from both sensitized and nonsensitized animals. Smaller contractions were also recorded upon the administration of bradykinin (1 μg/ml) and serotonin (10 to 100 μg/ml). The lack of a stimulant action of histamine in Dale and Gaddum's (*1*) experiments on the denervated hemidiaphragm of young kittens can be attributed to the lower sensitivity of cat tissues to this compound. Similar differences between species would account for the fact that denervated rat diaphragm does not respond either to histamine, serotonin, or bradykinin (*3*).

F. ALONSO-DEFLORIDA*
J. DEL CASTILLO
CARMEN C. GONZALEZ, V. SANCHEZ
Department of Pharmacology, School of Medicine, San Juan, Puerto Rico

References and Notes

1. H. H. Dale and J. H. Gaddum, *J. Physiol.* **70**, 109 (1930).
2. R. Miledi, in *Enzymes and Drug Action*, J. L. Mongar and A. V. S. de Reuck, Eds. (Churchill, London; Little, Brown, Boston, 1962).
3. K. D. Bhoola and M. Schachter, *J. Physiol.* **157**, 20P (1961).
4. Supported in part by grant 1-S01-FR-05119-01 from PHS.
* On leave of absence from the Department of Physiology, School of Medicine, National University of Mexico, Ciudad Universitaria, Mexico 20, D.F., Mexico.

20 November 1964

Primate Research and Systematics

The neglect of some basic aspects of primatology—in particular, taxonomy and zoogeography—may be illustrated by reference to two recent communications, one on mirror display in squirrel monkeys [P. D. MacLean, *Science* **146**, 950 (1964)], the other entitled "Primate biology: planning meeting" (L. Carmichael and A. J. Riopelle, *ibid.*, p. 1078).

MacLean identifies his animals as "squirrel monkeys (*Saimiri sciureus*)" and goes on to say that they "comprise several closely related species." Perhaps he meant subspecies. In any case, he distinguishes two kinds of male squirrel monkeys. One kind consistently displays an erect phallus to its reflection in a mirror and is marked by a facial pattern described as "Gothic." The other scores very low in phallic response to reflection and has a so-called "Roman" facial pattern. The author is unable to determine the taxonomic relationship between the two kinds of monkeys and can only speculate on their respective places of origin in tropical America. He does, however, provide a photograph of each facial type. Finally, the variable narcissistic tendencies of the monkeys lead him to observe that "It will be of interest to learn whether or not there has existed any environmental difference between the Gothic and Roman type monkeys in regard to ancestral exposure to reflecting pools and streams from overhanging boughs." It would, of course, be more germane to learn the history of the experimental animals from the time and place of capture to the time of the first laboratory experiment performed on them.

Any interpretation of MacLean's findings, assuming the unlikely absence of extraneous conditioning factors, depends on a knowledge of the genetic relationship between the two kinds of squirrel monkeys. Should they prove to be members of different clans of the same race, one explanation may apply. Should they represent different subspecies or different species, then other explanations may well be in order. Unfortunately, there is no modern taxonomic revision for squirrel monkeys, despite the fact that these animals are widely used in anatomical, physiological, medical, and behavioral research.

The second communication deals with proposed cooperative studies on primate biology by American and Japanese scientists. The authors list three areas for joint studies agreed upon by participants in the meeting. These are "(i) comparative studies of inter- and intra-species characteristics of primates, (ii) anatomical, physiological, and behavioral studies of primates, and (iii) studies of the care and diseases of free and captive primates." The first field of study holds great promise for biologists, but its scope appears to be limited to such primate characteristics as social organization, communication, vocalization, and interspecies ecology. There is no evident concern for the reliable determination of the *kind* of animal, that is, the species or subspecies, whose "characteristics," anatomy, diseases, and so on are under study. Basic research on the origin, evolution, and dispersal of primates seems to be even more remote from the objectives of the proposed program.

To many Japanese primatologists, systematic studies may be academic. There is one native species of macaque in Japan, and most primatological work in that country has been done on that animal. In contrast, American laboratories and those zoological gardens where biological research is conducted house primates representing virtually every living family, a majority of the genera, and scores, perhaps hundreds, of species and subspecies. Most experimentalists identify these primates by the trade or vernacular names passed on to them by animal dealers. The names used may be no more specific than lemur, monkey, or marmoset. The kinds of apes are generally recognized, but except for the orangutan, there is more confusion than certainty in distinguishing between what may be considered a species and what a race in gorillas, chimpanzees, and gibbons. The place of origin of an experimental animal is often recorded as Miami, New York, San Francisco, or whatever U.S. port of entry is shown on the bill of lading. Not often is the animal's true provenance more precisely known than as Africa, Asia, or South America.

Much of the carelessness, confusion, or indifference stems from an unawareness of the importance of taxonomy and zoogeography in the evaluation of data derived from wild animals of unknown genetic stock. Many scientists may regard their laboratory primates as nothing more than chemical or physiological containers of a particular tissue, organ, or system needed in research, or they may treat the animals as mere vehicles for biochemical or microbiological experiments. These scientists are laudably meticulous about what is put into these living media, what is removed from them, and how. Few, however, evince more than a passing interest in these captive containers or vehicles as identifiable organic elements of an ecosystem. Many existing laboratory colonies of primates used in highly specific research projects are stocked with one or more unidentified species or subspecies and undergo, through deaths and replacements, uncontrolled changes in their taxonomic content. These taxonomic turnovers extend to the microfauna and microflora living in and on the host species. It would be folly to believe that no alteration or distortion of data and experimental results is caused by this state of affairs.

Many scientific discoveries in the laboratory cannot be repeated, and others are lost for want of a reliable