

# **METHODS IN PHARMACOLOGY**

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**Volume 1**

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**Editor:**

**Arnold Schwartz**

# **METHODS IN PHARMACOLOGY**

***Volume 1***

*edited by* **ARNOLD SCHWARTZ**

Division of Myocardial Biology  
Baylor College of Medicine  
Houston, Texas 77025

**APPLETON-CENTURY-CROFTS**  
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# **Methods in Pharmacology**

***Volume 1***

***METHODS IN PHARMACOLOGY***

**a series of monographs  
edited by Arnold Schwartz**

## **Dedication**

This first volume of METHODS IN PHARMACOLOGY is respectfully dedicated to the memory of Sir Henry Hallett Dale (June 9, 1875 – July 23, 1968) whose monumental contributions to Pharmacology depended so much on the *variety* of methods he employed.

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## Preface

The death of Sir Henry Hallett Dale two years ago brought to a close an era which was characterized by tremendous pharmacologic advancements. During this period, the employment of what present investigators might consider "primitive" techniques yielded information which has become classic. For example, in a paper by Barger and Dale, published in 1910\*, one finds not only a beautiful example of a structure-activity study and suggestions of the importance of membranes, but also the concept of *specific receptor* sites introduced by the statement that "the relation of the receptive mechanism to the base (i.e., drug base) may well be one of solid solution of adsorption, and, therefore, more analogous to that of an enzyme to its substrate . . ." The search for drug receptors continues today at an accelerated pace. Hopefully, fundamental knowledge of receptors and the nature of drug-receptor interactions will lead to a rational approach to drug design.

In acquiring skills in the scientific approach to drug mechanism and evaluation, the modern pharmacologist, whether senior investigator or student, frequently is required to employ techniques which are characteristic of other biologic areas. However, it is not enough simply to be grounded in physiology or biochemistry. A pharmacologist, for example, is not "a biochemist who throws a drug or two in his flask," but is rather an individual who should approach a problem by using sound pharmacologic principles, as well as whatever biologic, physical, or chemical techniques that can assist in supplying the answer. It is becoming increasingly obvious that an understanding of the complexities of drug actions and toxicities is vital for the growth and welfare of our society. The design of better drugs to combat specific ailments is a major objective of health-oriented institutions. Never before has there been a greater need for excellent departments and institutes of pharmacology in medical colleges and in allied health schools.

The purpose of **METHODS IN PHARMACOLOGY** is to acquaint the student, as well as the professional investigator, with the multitude of proven techniques that can be employed in pursuit of the objectives mentioned above, and, in general, to provide the pharmacologist with methods to investigate the action of drugs on both

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\*Chemical structure and sympathomimetic action of amines. **J. Physiol.**, 41:19-59, 1910.

the organic and molecular levels. Some of the methods require only simple devices, while others utilize expensive equipment that may not be readily available. However, the theory, importance, and usefulness of each procedure is discussed in detail. The authors of each of the methods were selected because of their proven skill and competence in a particular area of pharmacology. The methods are written in a relatively simple, yet detailed, form so that, with the proper equipment and practice, an individual can produce meaningful data even if previously unacquainted with the general techniques. Sufficient references are included for further background study and documentation. Typical examples of results are also included for comparative purposes. Limitations and pitfalls are frequently given.

No attempt was made to cover the entire field of pharmacology. Certain specific topics were selected for this first volume, primarily to present a relatively wide scope of available techniques. Heavy emphasis is placed on cardiac tissues because cardiovascular pharmacology is probably among the most active of the subdisciplines. Editorial prejudice is freely admitted. It is expected that future volumes will include other important areas such as neuropharmacology, renal pharmacology, gastrointestinal pharmacology, and so forth.

The editor recognizes that *some* of the techniques in the volume appear, in one form or another, in other specialized volumes, as well as in published papers. Nonetheless, most of the techniques either have not been published or have not been presented in the detail expressed in this text.

***Arnold Schwartz***

# **Methods in Pharmacology**

***Volume 1***

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## CHAPTER 1

# Nerve and Nerve-Muscle Preparations (As Applied to Local Anesthetics)

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## INTRODUCTION

This chapter discusses both general and specific aspects of the testing and evaluation of local anesthetic agents. Previous reviews on the testing of local anesthetics (Carney, 1951; Weatherby, 1964) have described testing procedures with major emphasis on whole animal studies. In addition, other reviews have discussed methodology (Gray and Geddes, 1954; Adriani, 1960; de Jong and Wagman, 1963; Barlow, 1964; Truant and Takman, 1965; Turner, 1965). Some of these discussions are brief, others are specialized, and finally others do not stress quantitative methods sufficiently. This chapter will include in vivo, in vitro, toxicity, physical chemical, and analytical methods of evaluating local anesthetics. Whenever possible, quantitative techniques will be stressed. Clinical testing will not be mentioned, except for brief references in the discussion.

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The principles of evaluating local anesthetics are substantially similar to those required to evaluate any potentially useful drug. Therefore, the pharmacology, toxicology, and various aspects of the chemistry of the compound in question must be studied. The depth of these studies is determined by many factors, but in the main it will depend upon a judgment of the potential usefulness of the drug. The techniques and methods discussed in this chapter are intended to provide sufficient information so that the results from the studies will provide a sound basis for evaluating the potential of a given drug for clinical use. In that sense, the methods taken together are rather more than a simple screen. While the basic principles are general, the local anesthetics as a specific class of compounds require rather specific methods for their sound evaluation. This chapter deals with those specifics.

## IN VIVO METHODS

The main subdivisions of local anesthesia (also, called regional anesthesia) in clinical practice are (1) *conduction* anesthesia, (2) *infiltration* anesthesia, and (3) *surface* or *topical* anesthesia. Therefore, the methods that have been developed for use with whole animals are designed to test these three types of block. Each will be discussed in sequence.

### *Conduction Anesthesia*

Conduction (also block) anesthesia depends upon the application of a local anesthetic on a peripheral nerve trunk, a nerve plexus, or the spinal cord so that nerve impulses cease to propagate through the treated region. Experimental study of conduction anesthesia goes back to the last century when Corning (1885) produced spinal blocks in dogs with cocaine. Table 1 includes a survey of in vivo techniques used to evaluate conduction anesthesia in animals. It becomes apparent from the table that many tests in many animals have been worked out. Therefore, selection of a proper technique requires the consideration of many factors. By experience many laboratories have found that perineural block in small laboratory animals offers the advantages of simplicity, economy, and relatively good reproducibility. More specifically, we will discuss conduction anesthesia in the sciatic nerve of the albino rat.

Six animals in one cage is a convenient number to use. Ideally the animals should weigh about 125 to 175 grams. The animals are identified by clipping the ears in a conventional way. An assistant suspends the animals in a prone position by grasping the base of the tail and thoracic cage. The anesthetist extends a hind limb to its full length and locates the depression for needle insertion by palpation with the left index finger. The site of injection is the area under the skin at the junction of the *biceps femoris* and *gluteus maximus* muscles. The sciatic nerve is blocked in the midthigh region with 0.2 ml of the drug solution administered by a 24- to 25-gauge, ¼-inch needle attached to a 0.25-ml tuberculin syringe. This is

TABLE 1  
*A Survey of Methods of Evaluating In Vivo Local Anesthesia*

Method	Species	References
<i>CONDUCTION ANESTHESIA</i>		
Plexus anesthesia	Frog	Sollmann, 1918a Bülbring & Wajda, 1945
Spinal block	Frog	Sollmann & Hanzlik, 1928 Bieter et al., 1932
Sciatic block	Rat	Truant, 1958 Truant & Wiedling, 1958/1959 Åstrom & Persson, 1961b
Sciatic block	Guinea pig	Shackell, 1935 Luduena & Hoppe, 1952
Spinal block	Rabbit	Bieter et al., 1936 Luduena & Hoppe, 1951 Luduena, 1957
Peridural block	Cat	Sechzer, 1965 Truant, 1965
Spinal block	Cat	Sechzer, 1965
Spinal block	Dog	Dvorak & Manson, 1930
<i>INFILTRATION ANESTHESIA</i>		
Infiltration of tail	Mouse	Bianchi, 1956
Cutaneous wheals	Guinea pig	Büllbring & Wajda, 1945 Luduena & Hoppe, 1956
Cutaneous wheals	Dog	Meeker, 1925
<i>SURFACE ANESTHESIA</i>		
Cutaneous anesthesia	Frog	Sollmann, 1918b Munch et al., 1933
Corneal block	Guinea pig	MacIntosh & Work, 1941 Chance & Lobstein, 1944 Truant & Wiedling, 1958/1959
Corneal block	Rabbit	Sollmann, 1918c Rose, 1931 Sinha, 1936 Wiedling, 1952 Truant & Wiedling, 1958/1959
Sneeze reflex	Rabbit	Nieschulz et al., 1958 Åström & Persson, 1961b
Pubic reflex	Rabbit	Cited by Truant & Takman, 1965
Urethral block	Dog	Langston et al., 1967
<i>MISCELLANEOUS METHODS</i>		
Withdrawal reflex	Earthworm	Block et al., 1964 Suskevich et al., 1967
Mobility	Goldfish & tadpole	Adams et al., 1926 Meyer, 1937
Vocalization	Mouse	Jones et al., 1965
Pseudohernia	Guinea pig	Loewe, 1936
Vagus block	Rabbit	Copeland, 1924
Sciatic nerve— respiratory response	Rabbit	Schmitz & Loevenhart, 1924 Hirschfelder & Ridges, 1933
Corneal healing time	Rabbit	Castrén & Lavikainen, 1963