INTRODUCTION

The objective of this review is to focus on some of the important aspects of in vivo methods currently used in experimental animals to study pain. While using the animals to study pain, the investigator should safeguard their rights in accordance with the ethical values and prevailing laws. To study pain, it is unfortunately necessary to inflict a certain amount of pain. However, the degree of pain inflicted should not cause intense emotional reactions, indicative of prolonged agony. The experimental protocol should minimize the suffering of the animals and ensure that they are humanely treated.

THE MEANING OF “PAIN” IN HUMANS VERSUS EXPERIMENTAL ANIMALS

According to the International Association for the Study of Pain, pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage." This definition distinguishes the pain under examination from the "pain" (grief) of losing a loved one, the pain (disappointment) of unfulfilled expectation, or the pain (exasperation) of attending an interminable meeting. It also recognizes that pain can and frequently does arise in the absence of noxious stimuli. Pain is not a simple sensation caused by a specific stimulus but rather a complex reaction and experience with a multidimensional quality; it obviously varies widely among individuals and even in the same individual at different times. Painful stimuli also influence affective reactions that interact with the sensory components of pain. With these considerations, pain or noiception is viewed as a complex experience, comprising a sensory component referring to the qualitative sensory experience elicited by the stimulus and a reactive component that refers to the accompanying affective and emotional response. In experimental animals, it is not possible to obtain verbal reports. Also, it is not clear whether animals perceive pain in the same fashion as human subjects, and the pathways transmitting nociceptive messages, brain transmitters, and their receptors in animals are similar to those in humans. With reference to animal experimentation, it would be helpful to define pain in an operational sense based on stimulus conditions and observable responses. The term "painful" is usually used synonymously with the term "nociceptive" or "noxious," which means destructive or tissue damaging. Alternatively, the term "aversive" is frequently applied to stimuli that elicit behavioral responses to avoid stimulus conditions. "Nociception" is a better term to use in animals than "pain."

CONSIDERATIONS REGARDING NOCICEPTIVE STIMULI

A nociceptive stimulus must be carefully selected since there is a variety of stimuli, differing in applicability and limitations. None of the current techniques meets all the requirements of an ideal nociceptive stimulus. The parameters for the nociceptive stimulus must be quantifiable and controlled with precision in order to minimize variability of ex-
experimental results due to fluctuations in stimulus parameters; the stimulus used should simulate as far as possible natural conditions (1); the stimulus must be easily and frequently repeatable. This aspect is problematic because tissue damage alters the response by either sensitization or reduced sensitivity; further, repeated presentations of nociceptive stimuli lead to anticipatory avoidance learning, which interferes with the testing process (2). Therefore the right choice of a stimulus may frequently depend upon the exact nature of the experimental condition and the types of responses. Since different classes of analgesics vary in their mechanisms of pain relief, it is recommended not to rely on any one form of nociceptive test during the determination of analgesic efficacies.

RESPONSES TO NOCICEPTIVE STIMULI IN EXPERIMENTAL ANIMALS

Whereas humans can express and distinguish a wide variety of painful sensation, animals can display only autonomic and/or somatomotor disturbances. Somatomotor responses are most commonly employed in the experimental analyses of pain. Some of the somatomotor responses are the tail flick and writhing in the mouse, vocalization in the rat, etc. The tail-flick response can be elicited in chondrotoized animals and therefore involves polysynaptic reflexes; however, in normal circumstances it involves long supraspinal pathways (3). Nociceptive responses such as jumping in the hot-plate test, writhing to a chemical stimulus, and vocalization to an electrical stimulus require a high degree of sensory motor coordination. Repetent presentation of the nociceptive stimuli modifies the responses following local alterations (which might or might not be accompanied by noticeable tissue injury), recruitment, facilitation, inhibition, and/or conditioning. Jacob (4) has shown that with single exposure, either a high temperature of the hot plate or a long period of contact of the paws is needed to elicit a jump response; with repeated exposures, jump occurs at a much lower temperature of the hot plate and replaces the licking reactions ("occlusion"). These phenomena do not occur if the container is low and covered, as the animal learns that it is helpless ("learned helplessness"), and therefore they depend on the awareness of the environment; once acquired, these behavioral alterations resist extinction at least for 24 hr (memory). These phenomena can be partly reversed by electroshock or by extinction procedure by putting the mice repeatedly on the same apparatus, except not heating the plate. Conditioning might occur even in simple tests involving spinal reflexes when the animals are repeatedly tested at short intervals.

QUANTITATIVE DETERMINATION OF THE ACTIVITY OF ANALGESICS

Quantification of the activity of drugs differs from one test to another. Depending on whether or not the nociceptive stimulus is kept constant, the methods can be divided into two groups. In the first instance the activity of the drugs is related to the disappearance of responses. Graded responses can be used either by scoring (the intraarterial bradykinin test) or by measurable features (latencies in the hot-plate test and the number of abdominal contractions in the writhing test). In the second case the activity of the drug is related to the stimulus variations to elicit the responses. These variations might be related to the duration or the intensity of the stimulus or both. When keeping the intensity constant, one measures the reaction time. However, the major disadvantage here is to adopt a "cutoff time" which alters the statistical distribution and the calculations. If the duration of the stimulus is kept constant, one then measures the thresholds. In reality, the duration is rarely kept constant, since it increases as the threshold is measured. When it is really kept constant, repetition of the stimuli influences the results and interferes with the objective. When the time course of the effect is desired, it is necessary to use separate groups of animals instead of repeated exposure of the same animals at different time intervals to avoid the interference of learning.

SOME COMMONLY USED NOCICEPTIVE TESTS

A great variety of nociceptive tests is currently used, differing from each other by the nature of the stimuli, parameters, sites of application, nature of responses, quantitation, and apparatus. Objectively, depending upon the nature of the stimulus, they can be classified into chemical, electrical, mechanical, and thermal methods.

Chemically Induced Nociception

A variety of chemical agents has been employed to produce pain. The intraperitoneal administration of a noxious chemical substance to mice and rats produced peritoneal irritation, which elicits a writhing response. This response is unlearned and reflexive in nature. Each episode of writhing is characterized by internal rotation of the feet, sucking in of the belly, elongation of the body, arching of the back, rolling on one side and remaining still, or turning around and circling the cage (5). Many chemical irritants have been used, which include acetic acid (6,7), acetylcholine (8), alloxan (9), bradykinin (10), hydrochloric acid (11), hypertonic saline (12), lipoxidase (13), oxytocin (14), phenylquinone (15,16), and serotonin (17). Acetic acid and phenylbenzoquinone are the two most commonly used irritants. The use of phenylbenzoquinone is associated with problems of solubility, photosensitivity, and autooxidation.

Writhing can be abolished by evisceration, intraperitoneal application of procaine, spinal transection, and ablation of the cerebellum. Midbrain decerebration eliminates arching of the back and decortication does not affect the writhing response at all (5). Writhing is also prevented by electrical stimulation of the periaqueductal gray (12). One of the major drawbacks of this test is the great variation in individual sensitivity, when the number of writhings is considered (15). Atropine, adiphene, and dicyclomine are inactive in acetic acid-induced writhing (16), indicating that intestinal spasm is not involved in the production of writhing (18). Analgesic activity is present, if the latency to the first writhe is prolonged or the frequency of writhing is reduced. The writhing test has the advantage of simplicity and sensitivity to all clinically useful analgesics (19). Both the agonists and the antagonist agonists are readily detected in this assay (20). On the contrary, injection of the opioid antagonist naloxone increases the frequency of writhing in mice.