THE PHARMACOLOGY OF CHEMICAL IRRITATION*

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Chemicals used as irritants comprise one of the older forms of medication, and still enjoy a rather wide popularity. Originally plant and animal drugs such as mustard, volatile oils, tars, and cantharides were most commonly used, but more recently many pure chemicals such as methyl salicylate, salicylic acid, alcohol, and phenol have been extensively employed. In very recent years the progress of chemotherapy and physiotherapy has greatly reduced the clinical popularity of the chemical irritants although they maintain a significant place in self-medication. Almost simultaneously with this change in therapeutic importance there has arisen a widespread interest in the control of chemical irritation which appears as a side reaction following the contact of chemicals with the body in such varied roles as medication, cosmetic effect, industrial hazards, clothing, and food processing. When it is realized that the undesirable reaction may arise from the pure chemical or impurities, from chemicals used in processing, from solvent action on containers and other sources, the importance of the problem may be readily appreciated. Currently two types of irritation are recognized, primary irritation and allergic or sensitizing irritation, either of which may cause the symptoms of contact dermatitis. The following discussion is concerned with primary irritation although occasional reference to sensitization will be made. The essential difference lies in whether irritation arises from single or repeated exposure, although a chemical may exhibit both properties.

As has so frequently befallen our older scientific terms, the words "irritant" and "irritation" have had apended to them new meanings or connotations. These words originate from "irritare," to excite, and the original meaning of irritation was a condition of undue excitement, the stimulus necessary to the performance of a function, or the act of stimulating. An irritant, then, was any agent which caused or induced irritation. Such an agent might be chemical, mechanical, or electrical. From an academic point of view this remains the meaning of the terms,

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and it is also the sense in which a modern physiologist uses the term when he refers to the irritability of a cell, a tissue, or an organ. Thus he may study the irritability of a muscle under different conditions, using induced current as the stimulus or the irritant. Unfortunately the term has come to be applied to almost anything that affects the living cell, particularly if the effect is deleterious. It becomes apparent then, that to discuss the practical problem of chemical irritation it is necessary to first describe the term in more detail.

Certain fundamental biological principles form the basis for a consideration of irritation. First, the function of a cell or more complex biological unit may be changed quantitatively but not qualitatively; that is, we may increase, decrease, or stop the performance of a biological unit, but we cannot change the effect of that performance. Second, if the quantitative change is not sufficiently great to interfere with the nutrition or metabolism of the cell, the effect is reversible; that is, the function returns to normal when the irritant is removed. Finally, excessive irritation results in permanent, irreversible damage to the cell.

In pharmacological terminology, which in this case is the one commonly used by industry and the laity, a "stimulant" is an agent which increases function, a "depressant" is one which decreases function, and an "irritant" is an agent which reproduces the symptoms of inflammation. Even so, there is room for confusion and often heated academic debate because stimulants frequently cause depression after the stimulation, or in some cases even before, depression is often preceded briefly by stimulation, and small quantities of irritants may produce only stimulation or depression. Rarely could there be a topic more conducive to confusion. These conceptions do assist, however, in delineating the topic of chemical irritation to more narrow bounds for the purpose of discussion.

Technical criticism excepted, it may be pointed out that since the terms stimulation and depression refer to functional levels which may be altered, they apply to the systemic actions of highly specialized tissues or organs, while irritation might occur in less specialized tissue and applies to local actions resulting from direct contact. In general this is the case and we associate irritation with the less specialized and more accessible tissues such as the skin and mucous membrane.

The symptoms of irritation are easily recognized in the visible tissues. As classically described through the ages under the term of inflammation they consist of redness, heat, swelling, and pain. These are the cardinal symptoms of irritation and are often described under the impressive terms of rubor, calor, turgor, and dolor, respectively. Between these extremes of terminology are the more commonly used clinical terms such as hyperemia, erythema, thermal elevation, edema, conges-

tion, nociperception. Indulgence is asked for use of the common descriptive terms.

Elucidation of the pharmacology underlying these symptoms involves two phases, first the immediate biological phenomenon responsible for the symptoms, and next the pharmacodynamics by which the chemical initiates this phenomenon. In the case of redness the color is due to distension or dilation of the cutaneous vessels. This is the physiological response of the body seeking to remove the irritation by supplying additional blood to the area. In some instances this dilation is accomplished through the operation of the axon reflex, without participation of the central nervous system, although the latter also responds and is responsible for the reactions known as referred pain, counter-irritation, and central stimulation. In other cases the dilation is due to a direct injurious action on the vascular system. It should be pointed out here that the action is local and that when redness is the only symptom the blood is still entirely contained within the vessels, in contrast to the redness following bruises and the swelling accompanying more severe irritation.

The heat produced by local irritation is entirely relative to the normal skin temperature. Skin heat loss is regulated by the central nervous system on a systemic basis, therefore the increase in vascularity is not accompanied by a similar increase in heat loss and there is a resultant local increase in temperature.

As the vascular dilation becomes more severe the capillaries tend to become semipermeable permitting the extravasation of the blood serum into the extracellular spaces. If these exudates remain discrete no permanent tissue damage is done although swelling is apparent. If the exudates coalesce into large blisters there is tissue separation and repair is necessary. In view of the local character of the reaction there are seldom any systemic symptoms such as accompany the comparable but more generalized extravasation of shock. The pain of irritation is considered to be a combination of two factors, first a pulling or stretching of sensory nerves plus a direct pressure or pinching due to the swelling. Perhaps the more important mechanism is the direct irritation of the sensory nerves, for the pain is often replaced by local anesthesia due to damage of the nerve.

If the degree of irritation is not excessive it is possible to produce all the above symptoms without permanent injury to the tissue. If the irritation is excessive the cell is killed and a condition of irreversible injury exists. This is usually referred to as necrosis and forms a basis for evaluating the severity of irritation, although it is not necessarily one of the symptoms.

When we go one step back of the symptoms and attempt to explain irritation at the level of the cell we encounter a few apparently well-established facts, and a great number of uncertainties. We know, for example, that heavy metals in solu-

tion will precipitate the protein of the cell and thus destroy the cell, yet in weak solutions such a reaction may be regulated to produce an astringency, as when zinc sulfate is used in the eye. The irritation of caustic acids and alkalies may be explained in a similar manner. In the case of concentrated acids the withdrawal of water from the cell is also an important factor and we may generalize that the osmotic balance of the cell is quite sensitive. In clinical practice it is customary to adjust the tonicity of parenteral or injected solutions to that of the blood to avoid irritation.

In general the body can withstand a reasonable range of pH and it has been recently claimed that even the eye may tolerate a solution with pH as low as 5 if the osmotic pressure is controlled. It is generally accepted that solutions with pH outside the range of about 5 to 9 will be irritating to mucous membrane if not to intact skin.

This admittedly brief summary of the more obvious mechanisms of irritant action includes those which are most easily controlled chemically by adjustment of concentration, masking of ionization as in the case of the silver proteinates, buffering, or adjustment of osmotic pressure. So far as these factors are concerned it would be relatively easy to predict the irritant potential of a drug or formulation. There are, however, many chemicals, particularly the organics, whose mechanism of irritant action is not understood and cannot be predicted in advance, except in a general way. To further complicate the picture it is possible to increase the potency of a known irritant by the addition of another substance such as a wetting agent. To a large extent it is this latter group of chemicals which has made necessary the development of testing techniques to evaluate the irritant properties of chemicals by biological means.

In developing satisfactory testing technique the pharmacologist strives for as much simplicity as is consistent with the greatest possible reliability and quantitation. Even so, the tests developed require multiple subjects and a rather wide variety to meet the particular requirements of each problem. Those methods to be discussed are the experimental techniques used on laboratory animals prior to the clinical tests on human subjects.

Since the mechanism of irritant action is not clearly understood, the logical starting point in the development of methodology is the symptoms of irritation which have been discussed. In general it may be said that each of the several symptoms has been used as an end point in various methods with varying degrees of success. The rabbit and the guinea pig have come to be the most widely used animals, experience having shown that for primary irritation and sensitization there is a general agreement between the results in these animals and man. There is an occasional exception to this correlation but not enough to invalidate the experimental tech-

niques as screening measures prior to human patch testing.

We have seen that redness is usually the earliest visible symptom and it is widely used as a criterion. It is the one symptom which may be casually observed during the study of other phases of drug action in the routine pharmacological investigation. Important considerations in the evaluation of redness tests are the method of application, the concentration of the chemical, the site of application, and the relative degree of effect produced by a given exposure time. Water-soluble chemicals are usually applied in aqueous solution to the eye by instillation into the conjunctival sac, to the skin by wet dressings or intracutaneous injection. Preliminary range-finding tests are made to establish a suitable concentration for more detailed study. This leaves perhaps the most difficult problem of all, the evaluation of the effect. Various approaches to this problem have been described, but no one method is found to be universally satisfactory. The qualitative estimation is relatively simple since it might be considered an all or none reaction, either there is or is not redness. Of the quantitative methods the most widely used are measurement of the area of redness, application of an arbitrary scale for depth of redness such as pale pink, pink, etc., and a measurement of the duration of the reaction. Offhand it would appear that the measurement of area and of time would be the most satisfactory, but either or both of these cannot give an accurate index of the degree of action, for both are largely dependent upon the degree of vasodilation produced, which in turn is best measured by the color produced.

The heat produced by local application of an irritant is of questionable value as a measurement of the degree of action. This can be easily understood when it is remembered that the reaction is limited to the irritated area and that the measurement must be made at that site; it is not a systemic reaction which would be called a fever and could be measured by clinical methods. Although such techniques have been used, it is difficult to measure significant differences in the temperature of a small area of skin or in the eye. Normal skin temperature varies widely and rapidly with environmental temperature, muscular exertion, and excitement. Under ideally controlled conditions, which would be extremely difficult to achieve on a large scale in the laboratory, the maximum variation in temperature would be between the normal skin temperature and the body temperature as measured systemically. Aside from these practical obstacles there is the more fundamental objection that maximum temperature increase might be reached considerably sooner than maximum dilation. Therefore, while temperature measurement may have some specific advantages, it has not come into widespread use in the evaluation of irritation.

Swelling, or the production of

edema, is one of the most useful of the symptoms for quantitative estimation of irritation, and many different methods have been developed for its measurement. Here again, measurement of the area is perhaps most common and rapid, but is incomplete because it neglects the third dimension which is elevation. If area and elevation are both measured the volume can be computed and the evaluation is much more complete. In recent years two entirely different methods have been advocated for the measurement of the edema produced by irritation. In the first of these methods one eye of a rabbit is exposed to the irritant, which may be a vapor, liquid, or solid, for a definite period of time. Immediately after exposure the animal is sacrificed and both the normal and irritated eyelids are removed and accurately weighed. After drying at elevated temperatures both lids are again weighed. A comparison of the wet/dry weight of the two lids gives an estimate of the amount of edema produced. The second method makes use of a whealometer, which is essentially a diaphragm connected to a manometer. By recording the manometric readings from a plane surface of the body before and after irritation, the volume of the edema may be measured. These two methods are perhaps the most quantitative yet developed but their scope of usefulness is limited by the fact that the existing irritation must be sufficiently great to produce edema. They are of no value in estimating mild irritation. Another method for which quantitative accuracy is claimed is probably a measure of edema although the exact mechanism has not been delineated. In this method very small quantities of the irritant solution are injected subcutaneously into the ears of rabbits. After a period of time a solution of trypan blue is injected intravenously and in a relatively short time the dye tends to collect at the site of irritation. The depth of color, which within limited range is proportional to the degree of irritation, may be determined by appropriate scales. On the basis of available data this test seems to be somewhat more sensitive than most of the others for certain types of chemicals.

The last of the cardinal symptoms is pain, which to the average individual seems to be a very real entity. Unfortunately, however, pain is an extremely elusive phenomenon. A review of the methodology of pain measurement, with all its theoretical implications, would be beyond the scope of the present paper and would not be justified in view of the role played by pain in the study of irritation. While it is true that pain usually accompanies irritation, it cannot be taken as an accurate criterion, for, as has already been pointed out, the pain may be of only fleeting duration while the irritation may be extensive. From the practical standpoint too, the problems of accurate pain measurement even from standardized stimuli are extremely complex and much literature has been devoted to the

subject. While the problem is vital to the study of analgesic drugs, we find that in the study of relative irritation, pain plays only a minor role. In practice the amount of pain produced in the experimental animal is recorded by such relative terms as mild, moderate, severe, extreme, depending upon the visible reaction of the animal as reflected in blinking, pawing, phonation, etc.

A test which has special significance for compounds coming into contact with mucous membrane is based on the ciliary activity in the mouth of the frog. Following suitable preparation, it is possible to measure the speed of normal ciliary motility by timing the passage of a small object such as a particle of cork along the roof of the mouth. Bathing the ciliary bed with a test solution may accelerate, retard, or stop the motility. High dilutions of a given compound may cause acceleration while more concentrated solutions may permanently abolish the activity. It is a fascinating method and its implications are widespread.

These are the basic tests for pharmacological evaluation of irritation in the experimental animal. Each has been subjected to a great many variations and refinements involving intricate machines, elaborately controlled conditions and experimental designs, carefully described end points for the assignment of arbitrary values and statistical evaluation of the results. All of these factors help immeasurably in the study of irritation, but the original goal of simplicity consistent with reliability and quantitation is still far from sight. Each new chemical still presents its own problem and rarely, if ever, will a single technique suffice to properly evaluate it with respect to other irritants. From a practical standpoint, however, it is possible to outline a few general principles which will improve the reliability of the laboratory data.

First consideration should be given to the appropriateness of the technique to be used, the speed and economy with which they can be performed, and the number of different techniques to be applied. It is usually possible to get a preliminary screening with the simpler and more rapid tests, after which the more quantitative tests may be performed. Since there are many variable factors involved in any of the tests, including animal variation and human fallibility, it is advisable to select a suitable reference standard irritant wherever possible. This reference irritant might be one of the well-known irritant chemicals or, where product change or improvement is the objective, it should preferably be the present product if such is a stable, reproducible item. The important thought in favor of a reference is that it not only improves the reliability of the individual data, but acts as a "season skipper" by integrating all the data collected over a period of time into terms of a common denominator.

Interpretation of the data may usually be improved if it can be put

into numerical values. Two principal approaches are open to numerical evaluation, depending upon the circumstances. If only a limited number of compounds are to be studied, say six, they can be arranged numerically under each symptom or test in order of increasing severity of action. Totaling the score for each compound will then give its relative standing, the smallest score being the least irritant. The pitfalls of such a method should be obvious to any scientist, biological or otherwise. In the first place, it puts an equal value on each of the observed end points, which has been shown to be invalid. Perhaps the greatest error arises, however, from the tendency to consider the numerical ratio as an index to the relative *degree* of irritant potency whereas its very basis limits it to the relative standing of the compound with relation to others of the series. A further disadvantage is that, even with a reference irritant, such a scheme does not integrate data from different series of tests. It will place the compounds in each test with relation to the reference but does not place the compounds in the different series with relation to each other. If any appreciable volume of irritation tests are contemplated a more practical method of numerical evaluation is to assign a weighted score for each degree of irritation under each symptom or test. Such a method permits placing a total score for each observation which is in keeping with its relative significance. Totaling of the score again places the lowest score on the least irritating compound and permits integration of data obtained at different times either with relation to the reference or to the various test compounds.

With the exception of sclerosing agents the degree of irritation should be sufficiently great to produce clearly defined reactions but not severe enough to produce necrosis for, like other biological phenomena, minimal or maximal irritation cannot be evaluated on a graduated response basis. The data obtained, may be enhanced by using methods, animals, and scoring consistent with those in the literature This is not to condemn the search for new methods and improvements of the old, but to suggest that such efforts should be considered and encouraged as research and that the results thereof be published as such.

Observance of the above general principles, plus the exercise of good laboratory technique, should make it possible for the average laboratory technician to acquire considerable skill in the evaluation of relative irritant potential by routine methods. When such tests are more universally adopted as an essential part of new product development we may reasonably expect that there will be a concomitant improvement in the methods of testing, in the new products, and in the satisfaction that comes from having added one more important safeguard for the consuming public to whom we owe so much.