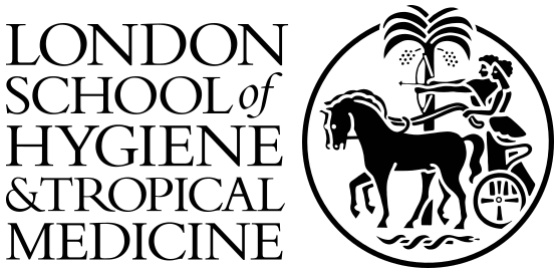

IN VIVO HUMAN OCULAR RESPONSES TO IRRITANT GASES

by

Jeffrey Ellis Coe, B.Sc., M.D.

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ABSTRACT

The purpose of this study was to define the *in vivo* human ocular response to irritant gases. To this end, several ocular response parameters--blink rate, surface temperature, and tear production--were measured before and after ocular sulphur dioxide exposure. Blink rate and ocular surface temperature were determined by conventional techniques, while tearing was measured by an original method based on the Schirmer test. Of these irritant responses, tearing proved to be the most consistent and quantifiable. In studies using both sulphur dioxide and ammonia, the tearing response was characterized in terms of speed of onset and resolution, dose vs. concentration dependence, and effect of stimulation of one eye on the unexposed, contralateral eye. The relationship between irritant gas concentration and tearing response was defined, and the threshold for the tearing response was found to be 5ppm. sulphur dioxide and 55ppm. ammonia. Finally, the afferent pathway for the tearing response was studied using a variety of physical and chemical blocking techniques: the ocular irritant response was found to be inhibited by impermeable corneal contact lenses, but was unaffected by topical anesthetics, beta-adrenergic antagonists, prostaglandin synthesis

inhibitors, and trigeminal spinal tractotomy (in individuals who had undergone this procedure as a treatment for trigeminal neuralgia). Taken together, these studies suggest that the in vivo human ocular irritant response is a neural reflex function of the trigeminal nerve initiated by specific corneal chemoreceptors, and that measurement of the tearing irritant response can be a useful adjunct to more conventional pulmonary techniques in determining threshold limit values for sensory irritants.

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PREFACE

Any study of the ocular effects of toxic environmental chemicals necessarially involves the disciplines of ophthalmology and occupational health. As such, it is the servant of two somewhat disparate masters. Both fields are highly specialized in approach and even in vocabulary. Because of this, at the outset I would like to beg the reader's indulgence. Although those in occupational health may well be mystified by the complexities of the eye's anterior chamber, and ophthalmologists by TLV's, PEL's, and the like, it is my hope that all will ultimately find something of interest and use in this investigation.

The research described here has its origin in Robert Douglas' 1975 Ph.D. thesis on the responses of the human lung to irritant gases. Hoping to compare the irritant responses of two different physiological systems, Douglas posed the seemingly straightforward question: "Is the human lung more sensitive than the eye to irritant gases?". To answer, he first determined the threshold for irritant-induced bronchoconstriction, and then compared it to the threshold for the subjective sensation of eye irritation. In general, the lung was found to be more sensitive than

the eye. But, recognizing the limitations of his methodology, Douglas expressed the hope that others would become interested in this problem and collect the objective eye threshold data required to make the eye-lung comparison truly meaningful.

The studies described in the following chapters stem from this simple question of more than a decade ago. At the outset, I naively believed that the sensitivity of the eye to irritants could be quickly and completely determined, but years have passed and the research has produced far more questions than answers. Along the way, however, much has been learned about the in vivo responses of the human eye to irritants. Like my friend and colleague Robert Douglas, I hope that this information will stimulate interest in this field and provide a firm basis for further inquiries into the interaction between the human body and the environment.

CHAPTER 1

INTRODUCTION

One of the greatest difficulties in the setting of occupational exposure standards is the lack of reliable information on human responses to environmental chemicals (Stokinger, 1969, Royal Society 1978, Thomas 1979, Henschler 1984). This problem is particularly acute for the eye. Chemical eye irritation, for example, has long been recognized as an important environmental health problem (NSPB 1949, NIOSH 1973), but relatively little is actually known about the nature of the human ocular irritant response. Few *in vivo* studies of this response have ever been undertaken and, because of the well documented structural differences between human and animal eyes, data from animal studies can be used only with great caution (Detwiler 1939, Rohen 1963, Bito and Klein 1981, Klein and Bito 1983).

The study of the human ocular irritant response has also been hampered by the lack of a generally accepted objective measuring technique. At present, most ocular irritant testing methods are subjective,

relying either upon trained observers grading corneal lesions in animals (Draize et al 1944, Federal Register 1961, Weltman 1965, Marzulli 1971), or on the subjective sensation of eye irritation reported by experienced human subjects exposed experimentally (Doyle et al 1961, Renzetti and Schuck 1961). However, being subjective, these techniques have proved difficult to standardize; there is significant variability in results between laboratories (Weil and Scala 1971, Burton 1972, Kaufman 1974, Conquet et al 1977), and intraspecies comparability of results has been repeatedly questioned (Buehler and Newmann 1964, Marzulli 1968, Beckley et al 1969, Ballantyne and Swanston 1972, 1973, Grant 1972, Royal Society 1978, Bito and Klein 1981, Klein and Bito 1982).

The purpose of this study was to systematically investigate the in vivo human ocular irritant response. To accomplish this it was first necessary to develop a sensitive and acceptable objective technique for measuring some aspect of the eye's response to chemical irritants. This method could then be used to begin to define the ocular irritant response in terms of function and, in so far as possible under in vivo conditions, anatomical structure.

The goals of the investigation were to answer the following questions:

1. How does the human eye recognize the presence of irritant gases? Is ocular irritant "perception" a function of a distinct "common chemical sense" as has been demonstrated in the human nose and lung (Keehle 1964, Montcrieff 1967, Sellick and Widdicombe 1971, Ulrich 1972), or is it simply another function of the already known ocular senses of pain and touch?

2. Where are chemoreceptors located in the human eye?

3. What are the afferent pathways for the human ocular irritant response?

4. How does the ocular irritant response compare with the previously characterized irritant responses of the human lung?

5. Is the measurement of the human ocular irritant response a useful adjunct to currently accepted respiratory methods (plethysmography, for example) in defining threshold limit values for irritants?

CHAPTER 2

REVIEW OF LITERATURE

Introduction

This chapter will briefly review the published studies on the human ocular response to chemical irritation. For convenience of discussion, the ocular irritant response will be divided into three stages: chemical perception (receptor structure and function), receptor-central nervous system pathways, and central nervous system integration of chemosensory information (including efferent pathways).

In considering the available literature, several general concerns should be kept in mind. First, as mentioned previously, the ocular irritant response has not been extensively studied so relatively little direct, experimental data is available. However, because much of what is known about the irritant responses of other organs agrees with that known about the eye, information from these sources will be brought into the discussion (particularly in reference to chemoreceptors). Secondly, very little information about the ocular irritant response comes from objective, *in vivo* human studies. The majority of the ocular studies reviewed here have involved animals and,

as mentioned in the Introduction, the use of such intraspecies comparisons can be questioned. Finally, most of the animal ocular studies have been highly unphysiological. For example, one common method for studying ocular responses to chemicals involves the use of an isolated corneal preparation (Tower 1940, Green and Tregar 1964). Although much interesting and important information has come from such studies, it is clear that these experimental conditions bear only the most tenuous relation to the "normal" human experience.

The "Common Chemical Sense"

It has been suggested that the perception of environmental chemicals may be a function of a separate "common chemical sense" (Keehle 1964, Montcrieff 1967). Originally coined by Parker (1922), this term refers to a "sixth" basic sense that, like the traditional chemical senses of taste and smell, responds to chemical rather than physical stimuli.

The common chemical sense can be experimentally distinguished from the senses of pain and touch, as well as from taste and smell. For example, it was early demonstrated (Sheldon 1909, Cole 1910, Crozier 1916) that animal limbs made insensitive to touch and/or pain by local anesthetics could still respond to

low doses of irritant chemicals. This response could be selectively exhausted by repeated irritant exposure, while pain and touch remained intact. Later studies of the human nose and mouth also showed distinct responses to irritants having neither taste nor smell (o-chlorobenzylidine malonitrile--CBMN--or chloracetophenone, for example), supporting the presence of a distinct chemical sense in man (Hubbard-Jones 1954, Keehle 1962, Montcrieff 1967, Alarie 1973, Doty 1975).

Although widely distributed over animal body surfaces, in man the common chemical sense is believed to be localized to mucus membranes and the lung (Keehle 1964). Stimulation of these chemosensitive areas generally produces a "protective" response--withdrawal of the head with nasal exposure, coughing with exposure of the upper respiratory tract, and bronchospasm with exposure of the lower respiratory tract. This response pattern has led to speculation that the common chemical sense may be a primitive, almost vestigial defense mechanism--perhaps being the primary chemical sense from which the more specialized chemical senses of taste and smell may have evolved (Keehle 1964).

Chemoreceptor Structure and Function

Irritant substances that stimulate the common chemical sense have several notable characteristics.

First, the nature of their effect on the target organ is either concentration- or dose-related: at low levels these substances provoke a characteristic, organ-specific irritant response (withdrawal, bronchoconstriction, tearing), while at high levels they produce pathological changes (Henderson and Haggard 1943). Expressed in a different way, perhaps the key characteristic of the irritants is that the target organ changes caused by low levels of these substances are completely reversible, while those caused by higher levels may be permanent. Finally, the irritant responses generally have a characteristic pattern, with low levels producing a rapid reaction that stops quickly and completely following irritant removal.

Chemicals fulfilling these criteria are commonly divided into three major groups on the basis of their chemical structure (Alarie 1973, Douglas 1980): thiol alkylating agents (primarily halogenated compounds like bromobenzylcyanide and chloracetophenone), dienophiles (chemicals with an ethylenic double bond like CBMN or acrolein), and organic chemicals containing trivalent arsenic.

From studies of the physical chemistry and molecular geometry of such substances, two major mechanisms of irritant-receptor interaction have been proposed, with irritants either reacting with

sulphydryl (-SH) groups or cleaving disulfide (S-S) bonds at some site on the receptor surface (Schmitt and Skow 1935, Dixon and Needham 1946, Mackworth 1948, Del Castillo et al 1951, Parker and Kharasch 1959, Alarie et al 1973). Because both -SH and S-S bonds are common in certain amino acids, it has been speculated that the receptor's "trigger" area might be a protien, possibly an enzyme (Csillik 1963, Karlin and Bartels 1966). Experimentally, in vitro interference with enzyme -SH groups and S-S bonds has been shown to alter cell permiability (Robinson 1966, Farah et al 1969) and block nerve cell depolarization (Karlin and Bartels 1966). Thus, if the chemoreceptor is viewed as a specialized nerve cell, it may be that irritants are "perceived" at the molecular level by interacting with one or more enzymes on the receptor surface, triggering an alteration in cell membrane permiability that generates an electrical response in the receptor and surrounding cells (Fatt and Katz 1953).

Specific histochemical evidence for the presence of -SH and S-S containing enzymes at suspected chemoreceptor sites has not yet been found. However, several in vitro studies support this model by showing accessible -SH or S-S groups near the binding sites of known cholinergic receptors (Del Castillo and Katz 1955, Karlin and Winnick 1968, Sobrino and Del Castillo

1972).

A number of authors have questioned the sulphydryl group-disulfide bond model of irritant action. For example, Peters (1963) correctly observed that the inhibition of thiol enzymes by irritants has never been observed *in vivo* and is irreversible *in vitro*, whereas one of the hallmarks of the irritant response is its rapid reversibility. While it is possible that other local conditions might make rapid reversal possible in the more complex *in vivo* environment, this argument is important and needs to be addressed by histochemists. Others have also noted that the model cannot account for the specificity of certain irritant agents: iodoacetate, for example, reacts strongly with sulphydryl groups but is not an irritant (Grant 1974), while Lewisite and the mustards react with sulphydryl groups but the response is slow (minutes to hours) in onset and produces only irreversible pathological changes rather than the reversible irritant response (Grant 1974).

As mentioned at the outset, few of the investigations of chemoreceptor structure have directly involved the eye. However, Green and Tregar's 1964 study of the irritant response in excised animal eye preparations is a noteworthy exception, and its findings also suggest that the actual ocular chemoreceptor

may be more complex than the model suggested above. Recording potentials from the ciliary nerve of a cat eye, Green and Tregar found that irritants like chloracetophenone and CBMN were self-blocking. Repeated applications of the same irritant diminished and, ultimately, extinguished the response, but during the period of self-blockade, the eye remained responsive to other irritants. In fact, only one substance, n-nonanoylvanillylamide (VAN, a capsaicin derivative), was capable of blocking responses to all other irritants during its own period of self-blockade. This finding has since been confirmed by others (Bynke 1983, Tullo et al 1983), and suggests that the ocular chemoreceptor must have a complex structure, possibly containing a number of active sites or having several entirely different chemical "triggering" mechanisms.

Microanatomic studies of the cornea in both animals and man (Tower 1940, Zander and Weddell 1951, Lele and Wedell 1956, 1959, Kitano 1957, Whitear 1960, Matsuda 1968) have clearly demonstrated an extensive network of unmyelinated nerve endings. These nerve endings appear to be structurally similar to the nasal unmyelinated nerve endings that Canua (1969) has identified as chemoreceptors. That these nerve endings may be the ocular chemoreceptors is also suggested by evidence that they are the only structures affected by

corneal application of capsaicin (Szolcsanyi et al 1975). Mosso and Kruger (1972, 1973) linked the corneal unmyelinated nerve endings to chemoreception in several neurophysiological studies in which the conduction velocity of impulses recorded in the trigeminal nerve following chemical corneal irritation was found to approximate that of unmyelinated nerves. Finally, the blocking effect of the biotoxins novesine and tetrodotoxin on the ocular response to corneally-applied irritants has been interpreted by Butler et al (1979) as evidence that the chemosensitive area of the rabbit cornea is quite superficial and composed of unmyelinated neurons.

The anatomical arrangement of the putative corneal chemoreceptors is unknown. Recording from isolated animal corneas, Mark and Maurice (1977), following the work of Tower (1940), found discreet receptor fields for pressure, temperature, and chemical stimuli. This study suggested the possibility of an underlying spatial arrangement of specific receptor types within the corneal epithelium. In an ultramicroscopic study, Kitano (1957) identified four different shapes of corneal epithelial nerve endings (free, "club"-shaped, retiform, and "blob"-shaped with a clear, 1-1.5μ "halo"), while Whitear (1960) later described three types of nerve endings (tree-like, fila-

ments, and beads). Though it is tempting to speculate that each shape of corneal nerve ending is associated with the perception of a specific stimulus (pressure, temperature, pain, chemicals, etc.), the differently shaped nerve endings appear to be randomly distributed throughout the corneal epithelium, forming no specific fields to correspond with Mark and Maurice's sensory maps.

In skin it has been found that superficial unmyelinated neurons can be specific for certain types of stimuli, with some fibers responsive only to irritant chemicals (Fallbrandt and Iggo 1961, Foster and Ramage 1981). Such specificity has never been shown for corneal sensory neurons and, recording from individual corneal afferent neurons, Lele and Weddell (1959) found that mechanical and thermal stimuli could excite the same fiber. Chemical irritants were not evaluated in this study, but the non-specificity of corneal afferents for physical stimuli does again suggest that corneal sensory reception may not be a simple process. Rather, the ocular "recognition" of specific environmental changes may involve complex local networks of neurons (Zander and Weddell 1951) or, perhaps, require extensive signal processing in the nuclei of the central nervous system.

Another possible mechanism for specificity in

corneal unmyelinated nerve endings has recently been suggested by studies of mouse taste perception. Lush (1981, New Scientist 1983) has argued that taste discrimination (a type of chemical specificity) is related to pore patterns in membranes covering the receptor nerve endings. Such a "molecular sieve" would selectively pass substances with certain chemical or physical properties (molecular size, shape, or polarity, for example) that would then contact the actual receptor site. This type of physical "recognition" mechanism for specific molecules could explain the specificity of the ocular chemical "sense" inspite of the non-specific distribution of the different shapes of corneal epithelial nerve endings and the apparent lack of specificity in the corneal unmyelinated nerves (Lele and Weddell 1959).

Another attractive aspect of Lush's theory is that the membrane sheathing the receptor would also serve a protective function, controlling the exposure of the nerve ending to potentially damaging environmental substances. This protection would be particularly important for chemical irritant receptors, because high levels of irritants are known to cause cellular damage. To date, however, such membranes have not been found, so confirmation will have to await further ultrastructural studies.

Although the mechanism of signal generation in the ocular chemoreceptors is not known, studies of mammalian eye inflammation by topically applied irritants (usually nitrogen mustard or liquid formaldehyde), strongly suggest the involvement of one or more neurotransmitter substances--similar to the "neurohumors" postulated by Jansco et al in 1968.

In response to nitrogen mustard application, the rabbit eye undergoes a predictable sequence of inflammatory changes, including miosis, hyperemia, increased intraocular pressure, and disruption of the blood-aqueous barrier with protien leakage into the aqueous humor (Jampol et al 1976, Tervo et al 1982, Bynke 1983). Further investigation has shown that the inflammatory response is actually biphasic. The first phase occurs within one hour and can be blocked, in whole or part, by retrobulbar injection of alcohol or novacaine (Davson and Huber 1950), section of the ophthalmic division of the trigeminal nerve (Jampol et al 1975), or experimentally-induced herpes simplex keratitis (Jampol et al 1975, Jampol et al 1976), but not by prostaglandin inhibitors like aspirin or indomethacin (Neufeld et al 1972). The second phase occurs after three to twelve hours, and can be blocked by systemic indomethacin, but not by denervation (Cole and Younger 1973, Podos and Becker 1973, Patterson and

Pfister 1975, Camras and Bito 1980a). From such studies it has been concluded that the later phase of the inflammatory response is mediated by prostaglandins (Camras and Bito 1980a), while the initial phase represents a neurogenic reflex (Jampol et al 1975).

The link between the neurotransmitter Substance P (SP) and the early phase of ocular inflammation has now been quite convincingly established. SP-containing neurons have been identified in the eyes of rabbits (Tervo et al 1981) and monkeys (Laties et al 1981), and in the corneas of rats (Miller et al 1981, Tervo et al 1981) and human beings (Tervo et al 1982a). These SP-containing neurons derive from the sole ocular sensory afferent, the trigeminal nerve (Brodin et al 1981, Tervo et al 1982b). In addition, SP has been shown to be released into the aqueous humor in response to eye irritation (Jampol et al 1976, Bill et al 1979, Camras and Bito 1980a), and direct ocular SP administration has been shown to produce an inflammatory response (miosis) in the rabbit eye (Bito et al 1982).

Further evidence for the role of SP in the early stage of ocular inflammation comes from studies using capsaicin (8-methyl-N-vanillyl-6-nonenamide). As mentioned when discussing the work of Green and Tregar (1964), capsaicin and its derivatives are irritants that are uniquely able to block the ocular response to

other chemical irritants. In an important study, Jessell et al (1978) linked SP to the chemoperception process by showing that capsaicin directly reduced the SP concentration in primary sensory neurons, like those believed to be the ocular chemoreceptors. The mechanism of action of capsaicin is still unknown, but it has recently been found to interfere with Nerve Growth Factor (NGF), a protien essential in the regulation of transmitter peptides like SP in intact sensory neurons (Otten et al 1983). Pre-treatment of the eye with capsaicin effectively blocks the initial, neural phase of the inflammatory response (Camras and Bito 1980a, Bynke 1983), confirming Green and Tregar's 1964 finding. Finally, in studies of experimental herpes simplex keratitis in mice, it has been possible to directly relate corneal sensitivity to SP levels (Metcalf 1982, Tullo et al 1983), again implying that this substance plays a central role in the mediation of ocular responses to the environment.

That the role of SP, though important, may not be direct or simple has been suggested by other studies apparently showing normal corneal sensitivity in rabbit eyes treated with SP-antagonists such as (D-Pro^{2}-D-$Trp^{7,9}$)-SP (Neuhuber et al 1981, Holmdahl et al 1981). However, these findings have recently been disputed (Mandahl and Bill 1983, Sasaoka et al 1984), because

this "antagonist" has actually been shown to be unpredictable, acting in different ways in different ocular tissues (Mandahl and Bill 1983).

In retrospect, it is not surprising that the definition of the primary transmitter substance for the ocular sensory response is complex, and that contradictory evidence exists. A recent review of research on peptidergenic neurons, for example, already lists more than twenty different peptides that are currently believed to be neurotransmitters (Hokfelt et al 1980), including a number of SP-related peptides that have been dubbed the "tachykinins" (Erspamer et al 1983). In view of this, it seems reasonable to speculate that SP is not the sole intermediate intraocular step in the first, neural phase of ocular inflammation. Already some research has suggested a possible role for bradykinin (Butler et al 1981, Couture and Cuello 1984) in what may be a chain or cascade of neurotransmitters involved in ocular chemical perception. And, in time, no doubt other intermediate transmitters such as histamine (Arvier 1977, Couture and Cuello 1984), 5-hydroxytryptamine (Arvier 1977), serotonin (Couture and Cuello 1984) and vasoactive intestinal peptide (Couture and Cuello 1984) will be identified--as well as preprohormones, prohormones, and peptide fragments of the implicated transmitters.

However, it is important to note again that the ocular studies cited here have all used chemicals sufficiently active (or at sufficient doses or concentrations) to produce the biphasic ocular inflammatory response. Because of this, their relevance to low level, transient chemical vapour ocular irritation--irritation that does not go on to produce the inflammatory response of miosis, increased intraocular pressure, hyperemia, and disruption of the blood-aqueous barrier--remains unknown.

Chemoreceptor-Central Nervous System Pathways

That the trigeminal nerve is the sole corneal sensory afferent pathway has been convincingly demonstrated anatomically (Magendie 1892, Bruce 1913, Rowbotham 1939, Rodgers 1953, Perkins 1957, Arvidson 1977, Butler and Hammond 1977, Mackie 1978, Butler et al 1979), physiologically (Lovick and Zbrozyna 1975, Kumado et al 1975, Accornero et al 1980), neurosurgically (Schimmelpfennig and Beuerman 1980, 1982, Lewis et al 1982), embryologically (Chan and Haschke 1982), and teratologically (van Bijsterveld 1968, Aleksic et al 1975, Mohandessan et al 1978). Because of this, it seems reasonable to assume that the trigeminal nerve is also the afferent pathway for the human ocular irritant

response. Although there has been no specific study of this chemosensory pathway, Green and Tregar's 1964 investigation in which the chemical irritant responses of the cat cornea were recorded from the ciliary nerve (a trigeminal afferent) supports the speculation that this nerve is the eye's primary chemical "sense" pathway. The human corneal innervation is similar to that of the cat and, in human beings, the sensory afferents for ocular touch, temperature, and pain are also carried by the ciliary nerve to the ophthalmic division of the trigeminal nerve (Mensher 1974, Alper 1975). Thus, although no direct human experimental evidence is available, by interference from animal models and other known human ocular sensations, the trigeminal nerve is undoubtedly the ocular chemical sense's afferent pathway.

Another, quite different source of human data also confirms that the trigeminal nerve is the ocular chemosensory afferent. Early in the century, ophthalmologists observed what has been called the "oculocardiac reflex", bradycardia associated with physical stimulation of the eye (Gillespie et al 1925, Kirsch et al 1957). The anatomical pathway for this reflex is the known connection between the trigeminal and vagus nerves at the level of the intracranial trigeminal nuclei (Kumado et al 1975, Dewar and Wishart 1976).

Little is known about the effect of ocular

chemical irritant exposure on heart rate, but two related studies have been published. First, in 1977 Ben David was unable to find any change in heart rate in response to ocular exposure to 168ppm. sulphur dioxide in human volunteer subjects. In animals, a 1971 study of the ocular effects of air pollutants by Basu et al did show a significant, albeit unexpected cardiac response. In this study, the eyes of anesthetized rabbits were exposed to a fixed-dose drop of acrolein while heart rate was monitored. All animals showed significant tachycardia, which was interpreted as a manifestation of the oculo-cardiac reflex, and might also be considered further evidence of the ocular common chemical sense. However, the finding of tachycardia rather than bradycardia was a surprise. In 1975, Kumado showed that direct electrical stimulation of the spinal trigeminal nucleus produced bradycardia similar to that seen with the oculo-cardiac reflex, and the irritant-induced tachycardia in Basu's study may be the result of a flaw in the experimental design with the response being more pharmacological (possibly from stimulation of pain receptors in addition to chemical receptors) than physiological. This type of study, though, does represent an interesting avenue of ocular research that, with modification (the use of topical anesthetics or irritants in gas form at concentrations

below the threshold for pain), might be more productive.

Central Nervous System Interconnections and Efferent Pathways

In both animals and human beings, the ophthalmic branch of the trigeminal nerve enters the central nervous system at the level of the medulla. This has been demonstrated anatomically and functionally in various species (Green et al 1957, Clark and Bowsher 1962, Kruger and Mitchell 1962), and also corresponds to the known pathways for other human ocular sensory afferents. Following entry into the human medulla, the ophthalmic afferents divide into two major branches (Nagano et al 1975): the first, carrying afferents for touch, pressure sensation, and two-point discrimination, ascends to the principal trigeminal nucleus, while the second, carrying afferents for pain and temperature sensation, descends to the spinal trigeminal nucleus. The detailed anatomy and function of these nuclei have been extensively studied (Wall and Taub 1962, Kerr 1966, Gordon et al 1961, Denny-Brown and Yanagisawa 1973, Kitihata et al 1973, Nagano et al 1975, Kirkpatrick and Kruger 1975) but, to date, no determination has been made as to which trigeminal nucleus receives ocular chemsensory information.

An important resource in studying the human

trigeminal nerve and its nuclei is the extensive literature on the surgical treatment of trigeminal neuralgia--a condition of unknown etiology marked by severe, recurrent pain in one or more of the trigeminal divisions. The neurosurgical treatments for this condition have grown increasingly sophisticated and specific (relieving pain while sparing other trigeminal functions such as touch), from the early peripheral nerve injections and sections, to partial ablation of the trigeminal (Gasserian) ganglion, section of the intracranial trigeminal tracts, and various "atraumatic" techniques of rhizolysis, microsurgical dissection, and medication (Loeser 1977).

Most interesting for the present study are the results of the trigeminal tractotomy procedures. As has previously been noted, the sensation of facial and ocular pain is related to the spinal trigeminal nucleus, while touch is a function of the principal sensory trigeminal nucleus. Interruption of the spinal trigeminal tract alone was found to give relief of trigeminal pain while leaving facial and ocular touch intact. Although this procedure proved to be less than ideal (see Chapter 5), it was widely performed beginning in the 1940's.

The outcome of trigeminal spinal tractotomy has been reviewed by a number of authors (Guidetti 1950,

Kugelberg 1952, Rushworth 1962, Janetta and Rand 1966, Hosobuchi and Rutkin 1971, Kilmov and Linke 1977), most of whom note that the corneal blink reflex is commonly lost (suggesting that this reflex is primarily related to pain sensation), and that although the ipsilateral eye becomes insensitive to pain it remains responsive to light touch. None of these reviews discussed whether the eyes of post-tractotomy patients remained sensitive to irritant chemicals, but the general acceptance of this surgical procedure in the 1940's and 1950's means that there still exists for study individuals in whom only the tract to the principal trigeminal sensory nucleus is intact.

Both of the major trigeminal nuclei have projections to the motor nucleus of the facial nerve (Nord and Kyler 1968, Nord and Ross 1973), and this relationship forms the basis for the corneal touch-blink reflex. Because the human lacrimal glands are also controlled by efferents from the facial nerve, it is likely that the medullary interconnection between the trigeminal and facial nerve nuclei also represents the primary pathway for the ocular responses to chemical irritants studied in the present investigation.

CHAPTER 3

METHOD DEVELOPMENT

Introduction

As mentioned in Chapter 1, there exists no generally accepted, objective method for measuring the responses of the human eye to irritant chemicals. Because of this, one of the primary goals of this study was to develop a reliable technique for quantifying the human ocular irritant response. To accomplish this, it was first necessary to select an appropriate ocular response for measurement.

A number of criteria were used in making this decision. First, the ocular response selected had to be accessible to measurement under *in vivo* conditions, and the measuring technique had to be acceptable to volunteer subjects. In addition, certain response characteristics were thought to be desirable, including 1. a rapid onset with irritant exposure, allowing close correlation of stimulus with response, 2. rapid resolution after exposure, permitting revalidation of pre-exposure levels, and 3. that the response be char-

acteristic--specific, in so far as possible, for chemical irritation or the specific irritant employed, decreasing the possibility of confounding from other, coincidental environmental changes.

From surveys of the subjective ocular responses to cigarette smoke and smog (Speer 1968, 1971, Weber et al 1976, Douglas 1975, Weber-Tschoop et al 1976, Okawada et al 1979, Shephard 1978) it was known that chemical eye irritation was commonly associated with tearing, increased blinking and, for certain irritants (acetaldehyde, for example), early conjunctival injection. Based on these reports and the constraints of available equipment and resources, an attempt was made to measure irritant gas-induced changes in blink rate, ocular surface temperature (as an indicator of increased blood flow in the conjunctiva and/or anterior chamber), and tearing.

Blink Rate

From the available literature it could not be determined whether the relationship between ocular irritation and blinking was direct (irritants stimulating the corneal blink reflex) or indirect (irritants stimulating tearing that, in turn, influenced blinking). A 1966 study by Schuck et al suggested that the measurement of blink rate could be a useful technique

for studying ocular irritation, but its sample size was small and it had never been repeated. In the ensuing years, techniques of great complexity had been used to study eyelid movement and tear flow during each blink (Doane 1980, 1981, Lemp and Weiler 1983). However, because only the rate of blinking was to be determined, Schuck's relatively simple technique was used in which the investigator observed the subject blinking and marked the occurrence of each blink on a moving chart recorder.

For comparison purposes, the "normal" rate of blinking, although individually variable, is 12-15 blinks per minute, with each blink lasting approximately fifty milliseconds (Duke-Elder 1971).

Ocular Surface Temperature

For the past half century there has been sporadic interest in the measurement of ocular surface temperature. As early as 1930, for example, using infrared thermometry, Zeiss found ocular surface temperature changes with inflammation; this response was later confirmed using a contact thermometer (Huber 1960) and improved radiometric techniques (Mapstone 1968a). In these studies it was found that unilateral ocular inflammation increased the surface temperature of the affected eye by up to 3^{o} C. compared to the contra-

lateral, unaffected eye (Mapstone 1968a). This striking change in the inflamed eye has been related to increased blood flow in the eye's anterior segment, with heat transmitted to the surface of the eye by the aqueous humor.

Both Zeiss (1930) and Mapstone (1968a) noted in passing that, in non-inflamed eyes, manipulation of the eye lids by the experimenter often stimulated tearing and was associated with temperature increases of up to 1° C. After the mechanical irritation stopped, the surface temperature returned to pre-irritation levels within fifteen minutes. For the present study, it was hoped that a similar temperature response might result from ocular irritant gas exposure. The study's working hypothesis was that irritants would increase the ocular surface temperature by stimulating tear production and/or increasing blood flow to the anterior segment and conjunctiva.

From published studies, the "normal" ocular surface temperature, again individually variable, ranges from 33-36° C. (Mapstone 1968a,b).

Tearing

Of the responses to be evaluated in the present study, tearing and its measurement have been most extensively studied. A major reason for this interest

is the existence of common clinical disorders of tearing (keratoconjunctivitis sicca, in particular). As a result, there has long been a specific need to measure tearing and to understand tear physiology and chemistry.

The best known and most commonly performed tearing measurement technique is the Schirmer test (1903). Used to determine the ability of the eye to respond to mechanical irritation, the test (in its simplest form) consists of a five minute tear collection using a relatively large (6 x 35 mm.) strip of filter paper inserted into the lower fornix. The amount of tears absorbed is determined by measuring the distance the tear margin has advanced up the strip.

Although useful in evaluating the eye's ability to respond to physical irritation, this technique would not allow measurement of the effects of chemical irritants or unstimulated tearing parameters. The Schirmer test, itself, is definitely irritating because the collecting strip directly contacts the lid margins, cilia, conjunctiva, and, occasionally, the cornea. That the collecting strips are irritating has long been apparent to patients undergoing the procedure but, more recently, has also been demonstrated by studies of the collected tears' chemical constituents (Van Haeringen and Glasius 1976, Berta 1983, Inada et al 1983, McGill et al 1983, Stuchell et al 1984). Finally, it is also

important that the Schirmer test's reliability has been repeatedly questioned: some investigators have reported consistent Schirmer test results over time for the same individual (Gifford et al 1943, Patton 1980, Prause et al 1982), but many others have found this tearing measurement to be highly variable (Wright and Meger 1962, Van Bijestervald 1969, Pinschmidt 1970, Herzberg et al 1973, Hanson et al 1975, Feldman and Wood 1979).

Because the Schirmer test cannot be used for studying characteristics of tearing other than its response to physical stimulation, a number of other tearing measurement techniques have been developed. The most precise determinations of unstimulated tear volume have come from slit-lamp fluorophotometry (Mishima et al 1966, Jordan and Baum 1980, Puffer 1980). Using this technique, for example, it has been determined that the "normal" tear volume is 7.0 ± 2µl.--1µl. in the pre-corneal tear film, 3µl. in the upper and lower menisci, and 3µl. in the fornices. Although highly variable, the unstimulated tear production rate is approximately 1.2µl./minute, with a range of from 0.5 to 2.2µl./minute.

As was the case with ocular surface temperature measurement, distinct irritant responses have been detected during the fluorophotometric tearing studies.

Mishima et al (1966), for example, noted that physical eye irritation during the measurement was associated with an almost immediate increase in tearing, often of more than one hundred percent. Although this technique would seem ideal for studying irritant gas-induced tearing, in practice it has several major drawbacks. First, to be reliable, fluorophotometry requires an elaborate (and expensive) instrument with considerable technical support (Wright 1984). Secondly, a number of studies have now shown that fluorescein, itself, is definitely an irritant (Mishima et al 1966, Jordan and Baum 1980, Lingelbach and Haberich 1982, Stodtmeister 1983, Mengher et al 1984). Thus, the use of this indicator might introduce a major confounding variable into any study of the ocular effects of environmental irritants.

In view of these problems, and because the filter paper methodology is relatively simple and well understood physically (Lamberts et al 1979, Prause et al 1982, Clinch et al 1983), the use of filter paper strips for tearing measurement remains attractive. Many modifications of the Schirmer test have been proposed, altering the tear collecting material (Schnitzler 1972, Kurihashi et al 1977, 1978, Farris et al 1981), site of tear collection (Kurihashi et al 1977, 1978), duration of collection (Tabak 1972, Farris

et al 1981), method of determining the amount of tears collected (Farris et al 1981) and, interestingly, even allowing Schirmer-type measurements to be made in other species (Harker 1970, Gelatt et al 1975).

Building on this work, a tearing measurement method was devised for the present study that used much smaller filter paper strips (3 x 10 mm.) placed under the caruncle--one of the least sensitive areas of the eye (Norn 1973). The strips were left in place for only fifteen seconds, and the amount of tears collected was determined by weight. Through these modifications of the Schirmer test, it was hoped to produce a sensitive, reliable, and acceptable measuring method that would avoid the potential masking of the chemical irritant response by physical eye irritation.

Methodological Considerations

Finally, a series of studies were undertaken to determine whether the irritant gas delivery and exposure system employed had any influence on the ocular irritant response. From previous studies it was known that tearing is extremely suceptible to environmental factors like wind or changes in light and temperature (Holly and Lemp 1977). Because of this it was necessary to insure that the wearing of the exposure goggles, flow of gas into the goggles, and the use of the

filter paper tear collecting strips were not, in themselves, irritating and capable of obscuring the chemical irritant response being studied.

Subjects and Methods

Subjects

This phase of the investigation involved 17 volunteer subjects (12 males and 5 females). Subjects ranged in age from twenty to fifty years.

All subjects were in good general health, and each was examined by a physician to insure that they were free from any eye disease or abnormality that might interfere with either the responses to be studied or the measurement techniques. The eye conditions of concern included congenital or post-surgical abnormalities of the eyelids or lacrimal system, acute or chronic conjunctivitis, acute or chronic eyelid infections, acute allergic eye changes, anterior chamber abnormalities of any etiology, keratoconjunctivitis sicca, and recent use of any ocular medication. No subjects reported any of these conditions and none were found on examination.

Prior to participation in the study, each subject was given a complete description and demonstration of the experimental procedures. Because the blink measurement method did not involve direct contact with the eye, it was not discussed with subjects at this preliminary stage. Written informed consent was obtained (Fig. 3.1) and was kept on file by

INFORMED CONSENT FORM

Ocular Responses to Irritant Gases and Vapours

I agree to be a subject in the experiments conducted by Dr Coe and Dr Douglas quantifying the effect of acute exposure of the eyes to irritant gases.

The experiments have been explained to me and I understand that I may withdraw at any time during the course of the experiments.

Name (block capitals):_____

Signature:..... Date:.....

Figure 3.1 Informed Consent Form

a member of the Department of Occupational Health, London School of Hygiene and Tropical Medicine (Dr. Robert Douglas).

A detailed presentation of the proposed experimental procedures was made to the Committee for the Control of Clinical Investigations and Experiments on Humans of the London School of Hygiene and Tropical Medicine. The written consent of the Committee was granted on June 3, 1980 (Fig. 3.2).

Methods

Irritant Gas Preparation

Sulphur dioxide for exposure was prepared by dilution of liquified BDH sulphur dioxide. Required amounts of this gas were drawn into a 50 ml. plastic syringe that had first been pre-conditioned by repeated (10x) filling with the source sulphur dioxide. This known volume of gas was then injected into an empty 200 l. Douglas bag that had been pre-conditioned by being filled with 200 ppm. sulphur dioxide for twenty-four hours. The gas was diluted to the required final concentration by the addition of compressed room air. The sulphur dioxide and room air were then mixed for five minutes by manual rolling and squeezing of the Douglas bag.

Figure 3.2

Notice From Committee for the Control of Clinical Investigations and Experiments on Humans

To: Dr. David Oakes

From: The Dean

3rd June, 1980.

COMMITTEE FOR THE CONTROL OF CLINICAL INVESTIGATIONS AND EXPERIMENTS ON HUMANS

I am pleased to inform you that the Committee has approved your application of 10th April 1980 for Dr. G. Coe to carry out a study of ocular responses to irritant gases and vapours.

C.F. Gordon Smith

The final sulphur dioxide concentration was determined by both Gastec sulphur dioxide sampling tubes and a Casella miniature SO_2 monitor. After preparation and concentration measurement, the diluted sulphur dioxide was allowed to stand for at least two hours to equilibrate to room temperature. From earlier studies, it was known that the concentration of sulphur dioxide prepared in this way remained stable for at least seventy-two hours (Douglas 1975). However, immediately prior to use, the sulphur dioxide concentration was rechecked with a sampling tube. In no case was the final pre-exposure concentration measurably different from that at the time of preparation.

Ammonia samples were also prepared by dilution of liquified BDH ammonia. A 10 ml. plastic syringe that had been pre-conditioned by repeated (10x) filling with the source ammonia was used to transfer required amounts of ammonia to the dilution vessel. For this gas, dilution in a Douglas bag was not technically feasible: in an earlier study, Douglas (1975) had found that, even after pre-conditioning, significant amounts of ammonia were lost by adsorption onto the walls of the mixing bag. Because of this, in the present study dilution and mixing were carried out in a 20 l. Pyrex bottle that had been pre-conditioned for two hours with 500 ppm. ammonia. After the bottle was

flushed with dried room air, the source ammonia was injected through the bottle neck. Ammonia and room air were mixed by a magnetically driven stirrer for at least thirty minutes, and samples of the gas for exposure were drawn from the bottle by a Reciprotor A/S 506-R Teflon-lined dry seal pump.

Final ammonia concentration was determined with a Gastec ammonia sampling tube. Through serial measurements, it was found that the concentration of ammonia prepared in this way remained stable for more than twenty four hours. In actual practice, all ammonia samples were used on the day of preparation, immediately following the equilibration period. Just prior to exposures, the final concentration of ammonia was rechecked by use of a sampling tube; in no case was this concentration found to be different from that determined at the time of preparation.

Exposure Procedure

In order to determine the usefulness of each irritant response measuring technique, the ocular response to be studied (weight of tears collected per fifteen seconds, blink rate, and surface temperature) was measured before and after a single five minute exposure to 50 ppm. sulphur dioxide (the highest concentration and exposure duration permitted by the

Committee for Control of Clinical Investigations and Experimentation on Human Beings).

For these exposures, sulphur dioxide was prepared as described above. Following dilution, the gas was transferred from the 200 l. preparation bag to a similarly pre-conditioned 2 l. polythene sample bag that could more conveniently be connected to the exposure goggles. Gas transfer was accomplished by joining the inlet tubes of the preparation and sample bags with a Teflon connector, and then manual compressing the preparation bag. No other tubing was introduced into the system and no mechanical pumps were employed.

Subjects were exposed to the irritant gas by means of a modified version of the exposure goggles designed by Douglas (1975, see Fig. 3.3). After pre-exposure measurements had been made (see below), subjects put on the goggles and a nose clamp, and closed their eyes. The test gas was allowed to enter the goggles for fifteen seconds, after which the subjects opened their eyes. For the remainder of the five minute exposure period, sulphur dioxide continued to flow into the goggles propelled by the elastic recoil of the sample bag's walls. The volume of each eyepiece of the exposure goggles was 10 ml.; by the end of the exposure period, the atmosphere within each eyepiece had changed approximately one hundred times. During

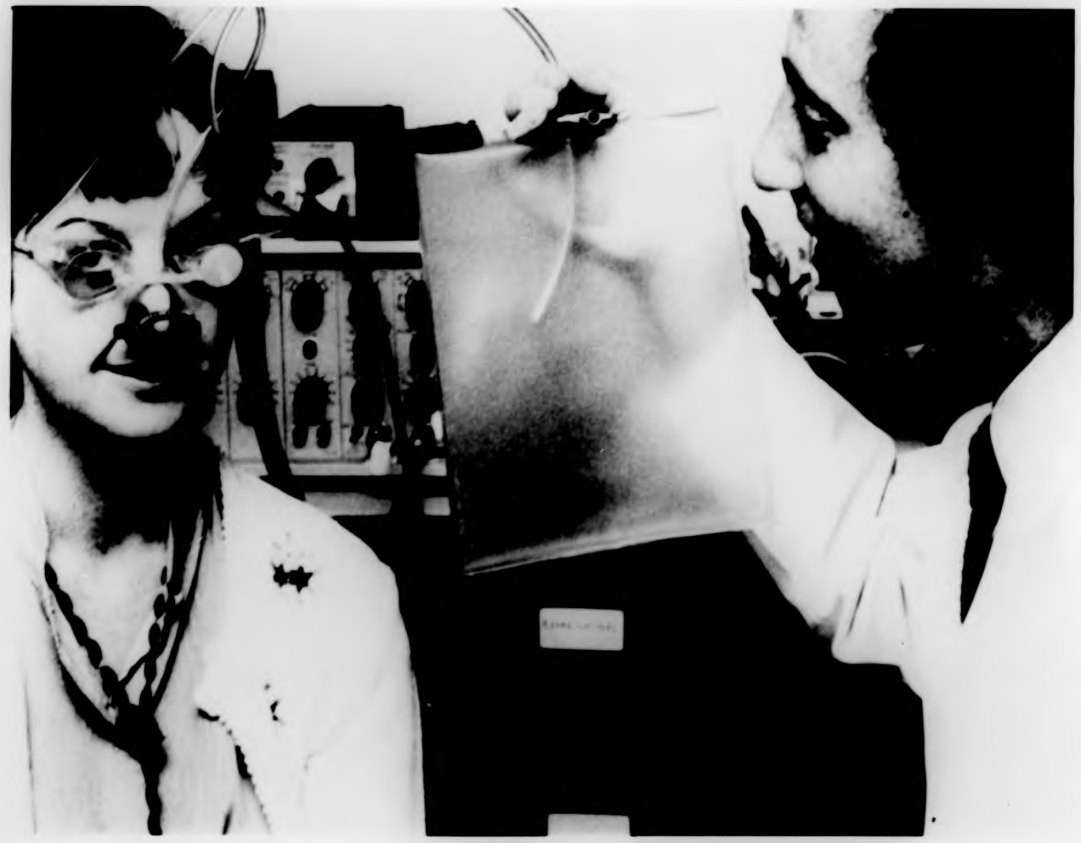


Fig 3.3 Irritant gas exposure system

the exposure, the observer encouraged the subjects to describe any ocular sensations they noted, and these comments were included in the experimental record.

Tearing Measurement Method Evaluation

Tearing was determined by measuring the change in weight of strips of Whatman No. 42 filter paper that had been inserted under the caruncle for fifteen seconds (Fig. 3.4). The filter paper strips were 3 mm. x 10 mm. and had an average dry weight of 0.1 mg. (Stanton Ultramatic UM-3 balance). Tearing measurements were made prior to exposure, immediately after the five minute exposure period, and at five and fifteen minutes after the exposure period. The mean of two or more measurements was used as the pre-exposure value; all other tearing determinations were based on one determination.

Because tearing is known to be influenced by environmental changes and anxiety, particular care was taken to define an acceptable range of "unstimulated", pre-exposure tearing. Again, from fluorophotometric studies cited earlier (Mishima et al 1966, Jordan and Baum 1980, Puffer 1980, Berta 1983), it was known that the "normal" tear volume in each eye is approximately 7µl. ± 2µl. and that unstimulated tear production is 0.5-2.2µl. per minute, or 0.1-0.5µl. per fifteen second

Fig 3.4 Tearing collection strip insertion

measurement period. Thus, for a non-irritated eye, it was assumed that the maximum amount of tears that could be collected in fifteen seconds would be approximately 9.5µl. or 9.5mg. (Berta 1983).

For the present study, the rounded value of 10mg. of tears collected per fifteen seconds was set as the upper limit of pre-exposure tearing. If the pre-exposure tearing measurement was greater than 10mg., the subject was asked to remain seated in the laboratory and tearing measurements were repeated every ten minutes until the tearing level fell to 10mg. or less. In no case did this acclimation, or "settling", period last more than thirty minutes, but the unstimulated tearing level of two subjects never fell below 10mg. Because of the consistency of these individuals' tearing measurements it was decided that the relatively high pre-exposure tearing levels probably represented a "normal" physiological varient, and these subjects were included in the study.

Blinking Measurement Method Evaluation

Each blink was observed by looking through the transparent eyepiece of the exposure goggles. The blinks were recorded by the experimenter manually closing a toggle switch, producing a 1.5 v. signal on the baseline of a Wantanabe chart recorder. Because

this measuring method did not involve direct contact with the eye, blink rate was measured prior to exposure and continuously during and after the exposure period. The number of blinks per fifteen seconds was later determined from the chart recording.

Ocular Surface Temperature Measurement Method Evaluation

Ocular surface temperature was measured by a Hermann I-R Thermometer (Model KT-12). In order to keep the instrument at a constant distance from the eye (1"), its lens was fitted into an aperture in one eyepiece of the exposure goggle. Output from the thermometer was amplified and recorded on a Wantanabe chart recorder. The thermometer was calibrated daily by means of an internal heat source, and was able to detect changes as small as 0.25° C. Again, because this measurement method did not involve direct contact with the eye, surface temperature measurements were made prior to exposure and continuously during and after the exposure period.

"Sham" Experiments

As mentioned previously, three "sham" experiments were undertaken to insure that the exposure and measuring techniques did not confound the responses

being studied.

First, the ocular irritation potential of the tearing measurement method itself was evaluated. In this study tearing was measured by the filter paper strip method every five minutes for forty-five minutes.

The second study sought to determine whether wearing the exposure goggles and nose clamp alone could stimulate tearing. Here, tearing was measured before and after wearing the goggles and nose clamp for five minutes, with no air flow or gas exposure.

The third study was to determine whether the flow of gases into the goggles could stimulate tearing. Following pre-exposure measurements, the subjects put on the exposure goggles and nose clamp. A 2 l. sample bag filled with room air was connected, and then allowed to flow into the goggles for five minutes, driven by the elastic recoil of the sample bag's walls. To mimic the actual gas exposure procedure, tearing was measured immediately post-"exposure" and at five and fifteen minutes post-"exposure". In this study, the nature of the gas (room air) was unknown to the subjects.

Sulphur Dioxide and Ammonia Dose-Response Study

In order to determine the relationship of the tearing response to irritant concentration, tearing

measurement studies were carried out using 2, 10, and 50 ppm. sulphur dioxide and 50, 100, and 250 ppm. ammonia. The logarithmic progression of concentrations was selected because it had been used in the subjective ocular studies of Douglas (1975). Only one concentration and gas was tested in any experimental session, and subjects were asked to tell the experimenter their subjective impressions of ocular sensation during the exposure period. Blank doses of room air were used during the sulphur dioxide exposure series.

General Experimental Design Considerations

Because tearing is sensitive to environmental and emotional changes, meaningful comparison of different subjects' pre-exposure tearing levels, or even the tearing levels of the same subject on different days, is not possible. As a consequence, for each exposure session subjects served as their own controls. Prompt (within fifteen minutes of exposure) return of tearing to pre-exposure levels was used as the indication of complete response resolution.

The exposures described in this thesis were all "single-blind" or "singly-masked", in that the gas concentrations were known to the experimenter but not to the subject. This experimental design was necessitated by the developmental nature of many of the tech-

niques and constraints of available personnel. It is well known that individuals have very different sensitivities to irritants (Sim and Pattle 1957, Frank 1964, Douglas 1975) and, particularly at the higher concentrations in the dose-response studies, some subjects were able to correctly guess the gas and approximate concentration. Because of this, it is possible that even the single-blind status some of the studies may be in doubt. The influence of these experimental design characteristics will be considered further in the Discussion section.

Data Analysis

The irritant gas exposures described in this thesis produced pairs of data (pre- and immediate post-exposure readings for different irritant gas concentrations) for each ocular response measured. These pairs were analyzed using simple statistical techniques (paired t-testing) to determine the significance of mean changes in tearing, blinking, and ocular surface temperature, as well as the significance of differences in the tearing response to different irritant gas concentrations. However, because the sample sizes were relatively small and it was not known whether the ocular responses conformed to a normal distribution, the data was also analyzed by the Wilcoxon signed-rank test, a distribution-free (non-parametric) method.

Results

Tearing Measurement

The results of the initial tearing measurement method evaluation study are shown in Table 3.1.

All twelve subjects demonstrated a significant ($p<0.01$) increase in tearing immediately after the five minute exposure to 50ppm. sulphur dioxide, when compared to pre-exposure levels. In all cases, by fifteen minutes post-exposure, tearing had returned to pre-exposure levels. The mean percentage increase in tearing was 149% ± 93%, with a range of 50-400%; if the extreme high and low values are excluded, the mean percentage increase in tearing was 125% ± 41%, with a range of 70-200%.

Because this initial evalution study suggested that the tearing measurement technique might be useful for studying the ocular irritant response, response studies were undertaken using 10 and 2ppm. sulphur dioxide and 250, 100, and 50ppm. ammonia. The results of these studies are shown in Tables 3.2 through 3.6. For 10ppm. sulphur dioxide, ten of the twelve subjects showed a significant ($p<0.01$) increase in tearing, with a mean percentage increase of 51% ± 33% and a range of 25-100%. Two subjects showed no change in tearing at this sulphur dioxide concentration. For 2ppm. sulphur dioxide, no subjects showed meaningful changes in tearing.

Table 3.1

Tear Production (mg. per 15 seconds) in Response to 50ppm. Sulphur Dioxide Exposure

	Tearing			
Subjects	Pre-Expos.	Post-Expos.	% Change	15' Post
1. J.C.	0.4	0.9	125	0.3
2. R.D.	0.8	1.5	88	0.7
3. B.G.	0.7	1.3	85	0.7
4. R.H	0.5	1.3	160	0.6
5. R.F.	0.2	1.0	400	0.4
6. L.W.	0.8	2.0	140	0.7
7. C.H	0.6	0.9	50	0.6
8. M.T.	0.6	1.8	200	0.5
9. J.M.	0.3	0.8	165	0.4
10. V.H.	1.0	1.7	70	1.1
11. P.A.	0.5	1.0	100	0.5
12. J.G.	0.6	1.8	200	0.4
		Mean	149%	
		S.D.	93%	

Table 3.2

Tear Production (mg. per 15 seconds) in Response to 10ppm. Sulphur Dioxide Exposure

	Tearing			
Subjects	Pre-Expos.	Post-Expos.	% Change	15' Post
1. J.C	0.6	0.9	50	0.5
2. R.D.	0.4	0.5	25	0.4
3. B.G.	0.8	1.2	50	0.7
4. R.H.	0.5	0.7	40	0.3
5. R.F	0.3	0.6	100	0.3
6. L.W.	0.6	1.2	100	0.6
7. C.H.	0.5	0.5	0	0.4
8. M.T.	0.7	0.9	28	0.5
9. J.M.	0.6	1.1	80	0.7
10. V.H.	1.2	1.3	8	1.1
11. P.A.	0.5	0.9	80	0.5
12. J.G.	0.4	0.6	50	0.3
			Mean 51%	
			S.D. 33%	

Table 3.3

Tear Production (mg. per 15 seconds) in Response to 2ppm. Sulphur Dioxide Exposure

	Tearing			
Subjects	Pre-Expos.	Post-Expos.	% Change	15' Post
1. J.C	0.5	0.5	0	0.4
2. R.D.	0.8	0.8	0	0.8
3. B.G.	0.9	0.8	-11	0.8
4. R.H.	0.7	0.7	0	0.6
5. R.F.	0.1	0.1	0	0.1
6. L.W	0.6	0.5	-15	0.5
7. C.H.	0.6	0.6	0	0.5
8. M.T.	0.5	0.6	20	0.6
9. J.M.	0.2	0.2	0	0.2
10. V.H.	1.0	1.1	10	1.0
11. P.A.	0.4	0.5	25	0.4
12. J.G.	0.6	0.6	0	0.6
			Mean 3%	
			S.D. 12%	

Table 3.4

Tear Production (mg. per 15 seconds) in Response to 250ppm. Ammonia Exposure

	Tearing			
Subjects	Pre-Expos.	Post-Expos.	% Change	15' Post
1. J.C.	0.4	1.4	250	0.4
2. R.D.	0.5	1.8	260	0.4
3. K.S.	0.5	1.5	200	0.5
4. J.D.	0.2	0.8	300	0.3
5. P.A.	0.4	1.7	325	0.3
6. R.M.	0.7	1.3	85	0.6
7. J.B.	0.6	2.1	250	0.8
8. P.B.	0.5	1.4	180	0.4
9. W.C.	1.1	2.0	90	1.2
10. B.B.	0.4	0.7	75	0.3
		Mean	202%	
		S.D.	92%	

Table 3.5

Tear Production (mg. per 15 seconds) in Response to 100ppm. Ammonia exposure

	Tearing			
Subjects	Pre-Expos.	Post-Expos.	% Change	15' Post
1. J.C.	0.6	0.9	50	0.5
2. R.D.	0.6	1.4	75	0.4
3. K.S.	0.3	0.6	100	0.3
4. J.D.	0.2	0.5	150	0.2
5. P.A.	0.5	1.5	200	0.3
6. R.M.	0.7	0.7	0	0.6
7. J.B.	0.6	1.4	150	0.6
8. P.B.	0.4	0.3	-25	0.3
9. W.C.	1.1	1.7	55	1.0
10. B.B.	0.7	1.1	57	0.7
		Mean	81%	
		S.D.	70%	

Table 3.6

Tear Production (mg. per 15 seconds) in Response to 50ppm. Ammonia Exposure

	Tearing			
Subject	Pre-Expos.	Post-Expos.	% Change	15' Post
1. J.C.	0.7	0.6	-14	0.5
2. R.D.	0.5	0.5	0	0.4
3. K.S.	0.8	0.7	-12	0.7
4. J.D.	0.3	0.3	0	0.3
5. P.A.	0.3	0.4	33	0.4
6. R.M.	0.6	0.5	-17	0.5
7. J.B.	0.4	0.4	0	0.5
8. P.B.	0.9	0.7	-22	0.6
9. W.C.	1.0	1.0	0	1.0
10. B.B.	0.5	0.5	0	0.4
			Mean -3%	
			S.D. 15%	

For 250ppm. ammonia, all ten subjects showed a significant ($p<0.01$) increase in tearing when compared with pre-exposure levels. The mean percentage increase in tearing for this concentration was 202% ± 45%, with a range of 75-325%. For 100ppm. ammonia, eight of ten subjects showed a significant increase in tearing; the mean percentage increase was 81% ± 70%, with a range of 55-200%. At this ammonia concentration, two subjects showed no change in tearing. Finally, for 50ppm. ammonia, no subjects showed any meaningful change in tearing following exposure.

The paired differences between each subject's percentage change in tearing with 50 and 10ppm. and 10 and 2ppm. of sulphur dioxide, and 250 and 100ppm. and 100 and 50ppm. of ammonia, were also significant at the 1% level using non-parametric methods.

The mean percentage changes in tearing in response to sulphur dioxide and ammonia plotted vs. log concentration are shown in Figs. 3.5 and 3.6. By extrapolation from these curves, the ocular tearing threshold for sulphur dioxide was found to be approximately 5ppm., while that for ammonia was approximately 55ppm.

The subjective ocular sensations reported by the subjects during these exposures were quite varied. At 50ppm. sulphur dioxide, several subjects (R.D., R.C.S., V.H.) described a slight "burning" or "irritation" that

FIG. 3.5: TEARING RESPONSE VS. LOG SULPHUR DIOXIDE CONCENTRATION

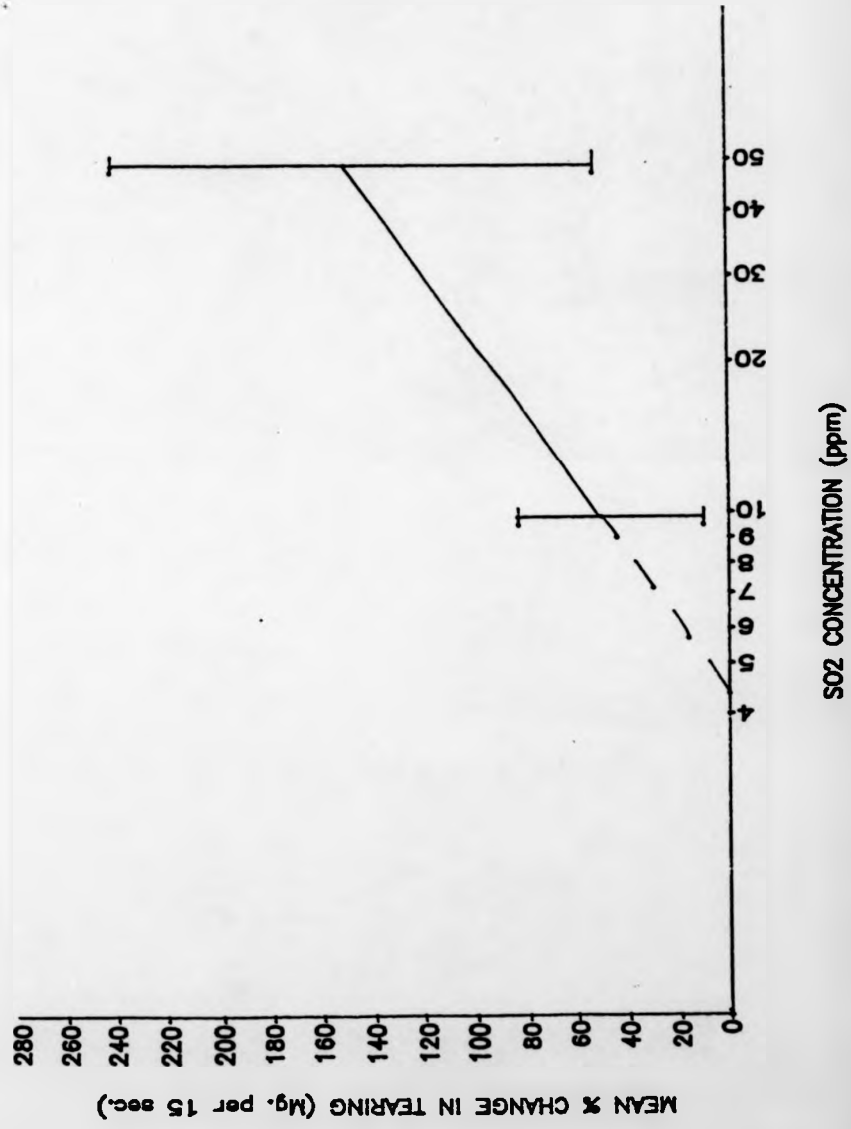
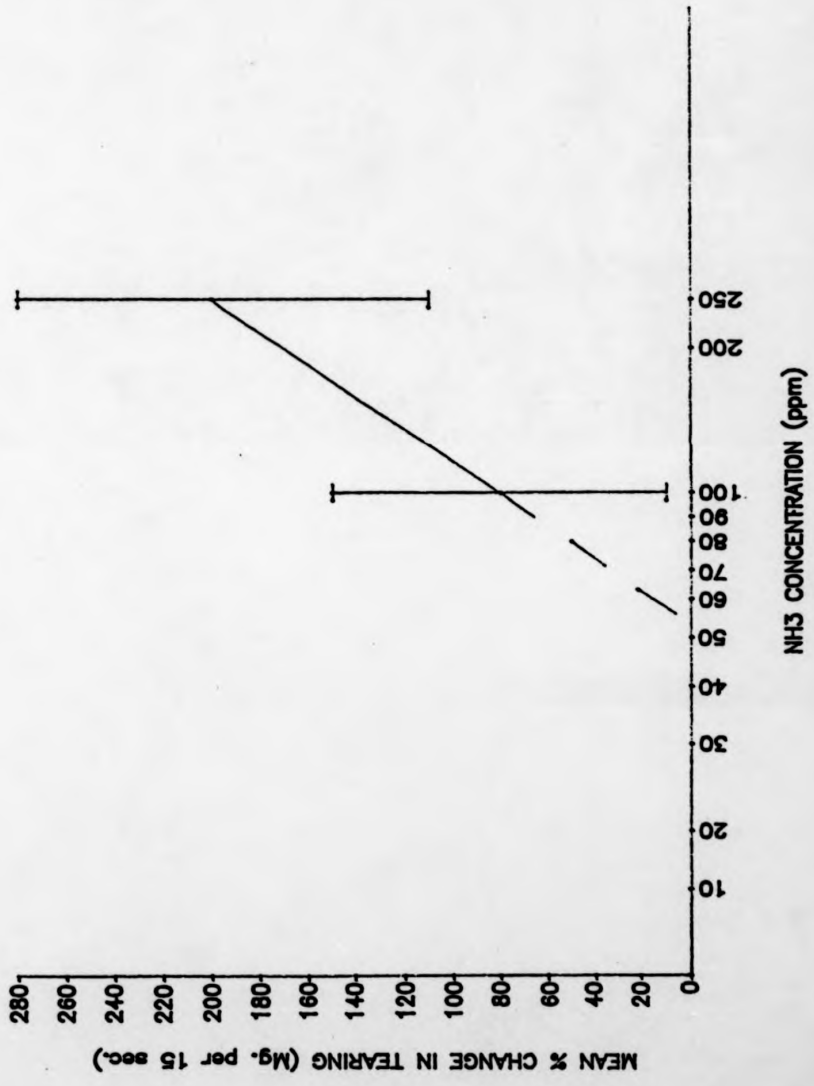


FIG. 36: TEARING RESPONSE VS. LOG AMMONIA CONCENTRATION



came on quickly and lasted for most of the exposure; they did not report any sensation of increased tearing or blinking. Other subjects reported a "cool" sensation about the eyes, but neither pain nor irritation. At 10 and 2ppm. of sulphur dioxide, no subjective symptoms were reported other than the occasional sensation of "coolness" that was probably due to gas flow through the exposure goggles.

Ammonia at 250ppm. was definitely perceived as irritant by eight of the ten subjects. Symptoms described during these exposures include a distinct "burning" sensation, marked "irritation", the sensation of tearing (R.M.), and a definite urge to blink more frequently (R.D.) associated with a rapid, regular blink rate of almost one blink per second. The two subjects (J.C., K.S.) who noted no subjective symptoms were both accustomed to wearing contact lenses (the lenses had been removed for at least one hour prior to exposure--see Chapter 4 for a further explanation of the exposure timing after contact lens wear). However, the objectively measured tearing responses of these two subjects were near the mean for this ammonia concentration (250% J.C., 200% K.S.) and the implications of this divergence between the objective tearing response and the subjective sensation of irritation will be considered in the Discussion section of this chapter

and of Chapter 5.

Blinking Measurement

The results of the blinking measurement study are shown in Table 3.7.

No meaningful change was found in frequency of blinking at either one or fifteen minutes after exposure to 50ppm. sulphur dioxide. The subjective ocular symptoms were much the same as those noted in the tearing measurement study. Two subjects (R.H., R.F.) noted mild irritation, but none complained of pain, tearing, or the sensation of increased blinking.

Because 50ppm. sulphur dioxide did not seem to be associated with any measurable change in the blink rate, the method was not evaluated with ammonia or lower concentrations of sulphur dioxide.

Ocular Surface Temperature Measurement

The results of the ocular surface temperature measurement study are shown in Table 3.8.

Immediately after the exposure, all subjects showed a 2-3^{o} increase in surface temperature that returned to pre-exposure levels within fifteen minutes of exposure. Unfortunately, although this change was highly significant ($p<0.01$), it was meaningless as an irritant response indicator. In an additional "sham" experiment in which subjects simply wore the exposure

Table 3.7

Blink Rate in Response to 50ppm.
Sulphur Dioxide Exposure

	Blinks Per 15 Seconds		
Subjects	Pre-Expos.	Post-Expos.	15' Post
1. J.C.	3	3	4
2. R.D.	4	3	4
3. B.G.	6	4	3
4. R.H.	4	5	3
5. R.F.	3	2	2
6. L.W.	2	3	3
7. J.M.	3	3	2
8. S.A.	5	5	3
Means	3.75	3.5	3.33
S.D.	1.30	1.07	1.07

Table 3.8

Ocular Surface Temperature (° C.) in Response to 50ppm. Sulphur Dioxide Exposure

	Temperature (° C.)			
Subjects	Pre-Expos.	Post-Expos.	15' Post	Goggles Alone
1. J.C.	34	36.5	35	36
2. R.D.	33.5	36	33	36
3. B.G.	32	35	32.5	35.5
4. R.H.	33	36	34	37
5. R.F.	34	37	34.5	35
6. L.W.	32	35	33	36
7. J.M.	33	35.5	33	35
8. C.H.	34.5	37	34	37
9. M.T.	33	36	32.5	37
Means	33.2	36	33.5	36.4
S.D.	0.87	0.71	0.87	0.50

goggles for five minutes without sulphur dioxide exposure, a similar, consistent temperature increase was also found. In view of this it seems likely that the ocular surface temperature change with exposure to 50ppm. sulphur dioxide was an artifact related to the exposure equipment or procedure.

Because exposure to 50ppm. sulphur dioxide revealed a methodological problem in the temperature measurement system rather than a meaningful irritant response, this technique was not used with other concentrations of sulphur dioxide or ammonia.

Equipment and Tearing Measurement Technique Evaluation Studies

The results of the three planned "sham" exposures are shown in Tables 3.9 through 3.11.

In all cases it was found that the experimental methods--the placement of filter paper strips under the caruncle, the wearing of the exposure goggles for five minutes, and air flow into the goggles at a rate equivalent to that of the test gases--caused no measurable change in tearing. Consequently, for the purposes of this study, it was concluded that these techniques were non-irritating.

Table 3.9

Evaluation of Irritation From Tearing Measurement

	Tear Production (mg. per 15 sec.)									
Subjects	0	5'	10'	15'	20'	25'	30'	35'	40'	45'
1. J.C.	0.4	0.4	0.3	0.5	0.5	0.4	0.3	0.4	0.5	0.3
2. B.G.	0.7	0.6	0.7	0.6	0.6	0.5	0.7	0.5	0.5	0.5
3. R.H.	0.8	0.9	0.8	0.9	0.7	0.7	0.7	0.9	0.8	0.7
4. M.T.	0.7	0.8	0.8	0.9	0.6	0.6	0.8	0.8	0.7	0.6
5. C.H.	0.5	0.5	0.7	0.7	0.6	0.7	0.7	0.5	0.5	0.6

Table 3.10

Irritant Potential of Exposure Goggles

Subjects	5' Pre-Goggles	1' Post-Goggles	15' Post-Goggles
1. J.C.	0.5	0.5	0.4
2. M.T.	0.7	0.6	0.5
3. C.H.	0.5	0.4	0.6
4. R.F.	0.3	0.4	0.4

Table 3.11

Irritant Potential of Air Flow Into Goggles

Subjects	Tearing (mg. per 15 sec.) 5' Pre-Exposure	1' Post-Exposure	15' Post-Exposure
1. J.C.	0.5	0.6	0.4
2. R.H.	0.6	0.5	0.6
3. C.H.	0.6	0.7	0.7
4. M.T.	0.8	0.6	0.5
5. S.A.	0.4	0.5	0.5

Discussion

In this section, only the method evalution studies will be discussed. The response curves undertaken as part of the evaluation of the tearing measurement method will be considered in Chapter 4, along with the results of other studies that further characterized the tearing irritant response.

Of the three irritant response measurement methods considered, only the measurement of tearing gave useful data. The other two methods were found to be faulty either because of technical difficulties or more fundamental problems relating to the response being measured.

Tearing

In theory, tearing seemed an almost ideal sensory response for measurement. Under reflex neurological control, its onset and termination were known to correlate well with stimulation (these characteristics of the tearing response will also be considered in Chapter 4). Although higher neural imput (emotions) and environmental factors (wind, light, temperature) can influence tearing, under controlled conditions the response was also relatively specific for irritation.

These characteristics of tearing were known from the ophthalmological literature but, prior to the pres-

ent study, few attempts had been made to objectively measure in vivo human chemical irritant-induced tearing changes. Several previous studies of tear constituents (protiens, electrolytes, enzymes) had used irritants such as crushed onion vapour (Thompson and Galliardo 1936, 1941, Brunish 1956, 1957), bromacetone (Krause 1959), and benzyl chloride (Regan 1950) to stimulate tearing. But, none of these studies specifically focused on tearing changes as an indication of ocular irritation. As mentioned earlier, the elegant fluorophotometric studies of Mishima et al (1966) and others clearly demonstrated a tearing irritant response, but the technique's complexity and use of fluorescein made it less than ideal for studying ocular responses to environmental chemicals.

It was against this background that the present study's filter paper measuring method was developed. As described earlier, the major changes between this method and the Schirmer test were: 1. decreased collecting strip size, 2. tear collection from a relatively insensitive area of the eye, 3. shortening of the collection period to fifteen seconds, and 4. weighing of the tears collected.

On the whole, these changes seem to have produced a useful tearing measurement technique. The "sham" experiment in which tearing was measured every

five minutes without irritant gas exposure showed no meaningful change in tearing over a longer period than that used in the experimental sessions, confirming that the technique, itself, was not measurably irritating. Further reassurance was provided by exposures to sub-threshold concentrations of sulphur dioxide (2ppm.) and ammonia (50ppm.), that showed no changes in tearing. Finally, Farris et al's 1981 study of unstimulated tearing also confirmed that such filter paper measuring methods need not be irritant. This earlier investigation used a technique rather similar to that of the present study: small filter paper strips were employed, collection was for an even briefer period (five seconds), and tearing was objectively determined by a method based on changes in electrical resistance. Farris et al's results will be considered in Chapter 4, but it should be noted here that this study's tearing measurement method was also reported to be non-irritant.

Some difficulties, however, were found with the current study's measuring technique. The major problem stemmed from the necessity of direct contact with the eye and the need for at least a moderate level of dexterity in measuring-strip placement. For example, inserting the strip too deeply occasionally produced a "burning" sensation in the area contacted and a transient increase in tearing. Alternatively, too shallow

an insertion could produce irregular tear collection and lead to the strip being dislodged by blinking. Both of these problems became less common with experience, but could be important sources of error for novice observers.

Not all subjects could tolerate the idea (or reality) of the collecting strip contacting their eye. For example, three potential subjects never became sufficiently comfortable with the measuring method to allow full participation in the study. These individuals pulled away from the observer while the strip was being inserted, had excessive reflex blinking, or obvious, psychological tearing (this could be recognized by its large volume compared to the tearing irritant response, as well as its association with other anxiety symptoms). Other apprehensive subjects, however, after observing a number of experimental sessions, became more comfortable with the technique and were able to participate in later phases of the study.

Thus, the study's subjects were a somewhat "selected" group, characterized by insensitivity to the prospect (or practice) of a paper strip contacting the medial corner of the eye. Elimination of this experimental design concern could only be achieved by the use of a completely different, non-invasive tearing measurement technique. However, systematic selection

bias seems unlikely because several initially anxious subjects went on to have relatively stable pre-exposure tearing levels once they grew comfortable with the procedure. In view of this it seems likely that the excessive blinking and tearing of the "discarded" subjects was a secondary, emotional alteration of the basic tearing response rather than another, distinctly different "normal" tearing pattern.

Because of the great range of individual sensitivity to touching the eye and, again, the effects of environmental factors, it was felt particularly important to carefully define an acceptable range for pre-exposure, unstimulated tearing. The limit of 10mg. per fifteen seconds seems to have been appropriate, and all subjects but two had pre-exposure levels below this limit. The two subjects with tearing levels at or above the limit were comfortable with the procedure and did not appear to be particularly anxious; these subjects' tearing responses were also similar in percentage change to those of the other subjects. Because of this, it was concluded that their unstimulated tearing level represented a normal varient and their results were included in the study.

Careful determination of pre-exposure tearing was also of concern because of the potentially large daily variation in the tearing response (Holly and Lemp

1977). However, in the "sham" experiments it was found that tearing could remain stable over at least forty-five minutes (longer than required for any of the exposures) under the specific conditions of the study--subjects seated in a quiet room with constant light and temperature, shielded from drafts, and familiar with the experimental procedure. In addition, in all cases tearing returned to approximate pre-exposure levels within fifteen minutes of exposure, again suggesting that no major shift in base-line had occurred. This study did confirm, though, the findings of Pinschmidt (1970) and others that there is little consistency in pre-exposure tearing levels from one day to the next.

Other sources of concern about the measuring technique proved to be unimportant. For example, it had been feared that there might be significant evaporative loss from the tear-laden paper strips, and it was to prevent this that closed plastic weighing chambers were employed. However, test weighings of the strip-containing chambers at one and five minutes after tear collection showed no loss and, in practice, all samples were weighed within one minute of collection. Of course, errors in the use of the balance are possible, but would be expected to decrease with observer experience.

Because tearing is an accessible, easily meas-

ured response that correlates well with irritation, its measurement remains attractive for further development. Ideally, the next generation of techniques should be non-invasive, possibly measuring evaporation (Rolando and Refojo 1983, Roland et al 1983) or reflectance (Lott and Cash 1973). Even invasive measuring techniques, however, can be further refined. For example, it should be possible to devise a single-step, direct-reading technique that would simplify the cumbersome two-step (collection and weighing) procedure used in the present study. Such a simplified method might be more acceptable than the current method because it would involve only one "instrument placement" (using a paper strip or, more likely, capillary tube or micro-electrode) and, perhaps building on the method of Farris et al (1981), measurement could be based on determination of electrical resistance.

Blinking

At the outset, the measurement of blink rate also seemed quite promising for quantification of the ocular irritant response. Like tearing, blinking was a well-known ocular protective reflex, and was related to tearing in that it functioned to spread the tear layer over the ocular surface (Doane 1980, 1981, Lemp and Weiler 1983). An early study had already shown blink

rate to be a useful indicator of the ocular response to photochemical smog constituents (Schuck et al 1966). And, finally, blinking appeared to have the great advantage of being relatively simply measured by non-invasive techniques.

Unfortunately, in the present study it was found that blink rate was not measurably affected by ocular exposure to 50ppm. sulphur dioxide. Although the specific reason for this lack of response is not known, both theoretical and methodological explanations can be suggested.

First, the highest concentration of sulphur dioxide used in this study (50ppm.) was still relatively low in terms of irritancy, not even consistently producing the subjective sensation of ocular irritation. The greater than 100% increases in tearing provoked by this concentration were still well below the thousand-fold changes found with emotional tearing, and were also within the capacity of the lacrimal drainage system to remove without overflowing (Holly and Lemp 1977, Lemp and Weiler 1983). Thus, at the maximum level of sulphur dioxide irritation used in this study, it is unlikely that the menisci would enlarge enough to necessitate increases in blinking for the preservation of clear vision. In contrast, all of Schuck et al's subjects reported distinct eye irritation and the sen-

sation of increased tearing, although no gross tearing was observed. In view of the lesser intensity of irritation in the present study, it may be that blink rate can only be used as an indicator of severe eye irritation and is not useful at lower irritant concentrations.

The response of one subject (R.D.) to 250ppm. ammonia, however, suggests that the threshold for blinking and tearing may not be identical. By the time of this exposure, tearing measurement had already been selected as the study's primary investigational technique and blink rate was not being regularly recorded. But, with 250ppm. ammonia--a concentration most subjects found to be only moderately irritant--this subject reported severe irritation and showed a marked increase in blink rate (to almost one blink per second). Curiously, this subject's measured increase in tearing was approximately 250%, a response near the mean for all subjects (202% ± 92%). In this case, then, the increase in blink rate was almost independent of the increase in tearing, a phenomenon also seen in one of Schuck et al's subjects. In view of the wide range of sensitivity to irritants, any single, unusual, extreme reaction to an irritant is not particularly surprising, but it does suggest that studying blink rate with higher irritant concentrations might be

useful, though the relationship between blink rate and tearing may not be simple.

In the present study a major technical problem was found in the relatively simple blink measuring technique. As was the case with tearing, the normal, reflex control of blinking can easily be overridden by imputs from higher neural centers. For tearing these influences could be controlled, but for blinking this was not possible. Almost anything that drew the subjects' attention to their blinking tended to inhibit the response. Unfortunately, a number of subjects quickly realized that each blink was being counted by the way in which they were being observed or the sound made by the chart recorder pen as each blink was marked. Aware of their blinking, these subjects brought the reflex under conscious control, and the blink rate results were not meaningful.

Techniques for unobtrusive blink recording should not be difficult to develop. Among promising approaches might be a simplified version of the cine technique of Lemp and Weiler (1983) or, perhaps more conveniently, using videotape to record each session.

Another possible technique might involve the infrared temperature measuring technique also used in the present study. The measurement of ocular surface temperature will be discussed below but, with regard to

blinking, a temperature difference of 1-1.5° C. was found between the relatively warm external surface of the upper eyelid and the cooler surface of the globe. This temperature difference was similar to that reported earlier by Mapstone (1968a,b). The current study's infrared thermometer was able to detect lid closure during slow, conscious blinking, but did not have a sufficiently rapid transient time to respond to the temperature change during a "normal" blink. Other infrared thermometers with more rapid transient responses are commercially available and would make a silent, temperature-based blink measurement system feasible. Although blink rate is probably a secondary, tearing-dependent irritant response, it should be reevaluated using a less "psychologically invasive" system before being abandoned as an indicator of ocular irritation.

Ocular Surface Temperature

Ocular temperature was measured in this study because it was speculated that irritant gas exposure might produce detectable temperature changes at the eye's surface. In 1975, for example, Douglas photographically documented marked conjunctival injection in response to acetaldehyde exposure, and it seemed likely that this increase in superficial blood flow would increase ocular surface temperature. In addition, it

was already known that reflex tearing increased ocular surface temperature: Mapstone (1968b), for example, measured and poetically described "the hot tears secreted in the relatively stable thermal environment of the superior fornix seeping across the surface of the colder cornea". As mentioned earlier, Mapstone noted this irritant-induced, tearing-related temperature change but never specifically focused on this process. His only interest was in developing a temperature measuring instrument and defining "normal" ocular temperature parameters; in consequence, he saw the irritant-induced changes only as something of a nuisance, necessitating a delay of several minutes to allow for reequilibration before more base-line measurements could be made. Finally, another potential influence on ocular temperature was thought to be evaporation, a cooling process that might offset the temperature increases caused by vasodilation and tearing (Rolando et al 1983, Rolando and Refojo 1983).

Unfortunately, the present study's exposure system seriously interfered with ocular temperature measurement, so irritant-induced changes in ocular surface temperature could not be satisfactorily determined. As seen in Table 3.8, the exposure system was consistently associated with a 2-3^{o} C. increase in ocular surface temperature regardless of the presence

of irritant gases. This local "heating" was almost certainly due to direct conduction of facial heat from the eye, eyelid, and forehead to the exposure goggle eyepiece. The study's exposure goggles were modified plastic swimming goggles (a common source for this piece of experimental equipment--see, for example, Jauregui and Fatt 1977), that were tightly applied to prevent escape of the irritant gas from around the eyes and dilution of the sample gas by room air. However, this tight "seal" also confounded this portion of the study by trapping facial heat in the eyepiece and producing the marked temperature increase.

From the "sham" experiments and sulphur dioxide and ammonia exposures at concentrations below the tearing threshold, it is known that the goggle-related temperature increase did not affect the tearing response. However, it did completely mask any possible irritant-induced change in ocular surface temperature. Following removal of the goggles, the ocular surface temperature returned to pre-exposure (or pre-goggle application) levels within three to four minutes. Temperature measurements made after this reequilibration period did not show any characteristic irritant-induced response pattern, and in later studies (see Chapter 4) it was found that by this time the irritant response had largely abated.

It is also interesting to note that no significant conjunctival injection was ever noted during this study. This observation may simply be another example of the insensitivity of such subjective criteria (similar to the finding that subjects could not perceive greater than 100% increases in tearing). It is also possible that vasodilation is only a late, slow component of the irritant response, or occurs only with high irritant concentrations, high total doses, or with certain specific irritants (such as acetaldehyde).

The success of the tearing measurement method decreased interest in solving the temperature measurement system's technical problems. But, in retrospect, making a practical instrument for measuring ocular temperature should not be difficult. Two specific changes would be helpful. First, in a later phase of this investigation (see Chapter 4), it was found that there was a definite contralateral tearing response with ocular irritation--that irritation of only one eye produced a tearing response in the opposite, unstimulated eye. The adoption of this procedure (using a single exposure eyepiece) would allow the opposite eye to remain at room temperature and avoid the masking effect of eyepiece heating. In the actual exposure technique used to study the contralateral eye tearing response, both eyes were encased in separated eyepieces

(one eye was exposed while the other eyepiece shielded the opposite eye from exposure to escaping gas); but, with care and a well-fitting "exposure" eyepiece, the "isolation" eyepiece might be unnecessary, and surface temperature measurements could then simply be made from the contralateral, unshielded eye.

Secondly, the sensitivity of the temperature measurement system probably could be improved by modification of the instrument's lens to allow focusing on a small (perhaps several square millimeter) section of the conjunctiva. The thermometer used in this study could not be focused in this way and recorded from the entire visible surface of the eye. Much of this large field is occupied by the cornea, an avascular structure. Thus, focusing on a vascular conjunctival field (possibly superior and lateral to the cornea, closer to the lacrimal gland itself) might increase the chance of detecting a temperature response from irritant-induced vasodilation or tearing.

Further work on ocular surface temperature measurement should be undertaken because, of the three measuring techniques, it still seems to have the greatist potential for the development of a non-invasive instrument. Using a modified television camera with infrared recording capability, for example, it might be possible to collect and store such ocular temperature

measurements on videotape for later analysis. Simple, sensitive, hand-held, infrared-sensing ophthalmological instruments have been available for a number of years (Mapstone 1968a,b) so this type of methodological development should be well within the realm of current technology.

CHAPTER 4

THE OCULAR IRRITANT RESPONSE

Introduction

In the studies discussed in this Chapter, the tearing measurement method was used to begin to define the *in vivo* human ocular irritant response. The response characteristics studied include: 1. the shape of the response curve, 2. the presence of a "contralateral" response (does the irritation of one eye produce tearing in the opposite eye?), and 3. the relationship of the tearing response to irritant dose and/or concentration. These characteristics were chosen because they allow the tearing response to be compared to the subjective ocular irritant responses as well as objective studies of the pulmonary irritant response. In addition, in this chapter the sulphur dioxide and ammonia response curves described previously will be considered.

Subjects and Methods

Subjects

Ten volunteer subjects (seven males, three females) participated in this phase of the study. The characteristics and criteria for selection of these subjects were as described in Chapter 3.

Methods

Three separate studies were undertaken to characterize the ocular tearing response:

1. Determination of the effect of sulphur dioxide exposure of one eye on the tearing response of the contralateral, unexposed eye.

2. Definition of the sulphur dioxide tearing response in terms of speed of onset, shape of the response curve, and speed of resolution following removal of the irritant.

3. Determination of whether the ocular tearing response to sulphur dioxide was dose or concentration dependent.

Irritant gas preparation, tearing measurement, and basic exposure procedure were as described in Chapter 3. Changes in the technique to allow unilateral exposure and rapid tearing determination are detailed below.

Definition of the Contralateral Eye Tearing Response to Sulphur Dioxide

For this study, the two eyepieces of the exposure goggles were separated by clamping shut the interconnecting tubing. In this way, irritant gas flow could be selectively directed to only one eyepiece; the opposite eyepiece was used to isolate the contralateral eye and prevent its accidental exposure to the test gases. The "exposure" eye was then exposed to 50ppm. sulphur dioxide for five minutes. Tearing measurements were made from both the exposed and isolated eye prior to exposure, immediately after exposure, and at five and fifteen minutes after exposure.

Definition of the Tearing Irritant Response Pattern

This study employed the separated eyepiece exposure system described above. The irritant gas was again 50ppm. sulphur dioxide. Tearing was measured in the isolated, unexposed eye prior to exposure and then as rapidly as possible during the five minute period of exposure of the contralateral eye. Following the exposure period, tearing measurements were continued until tearing had returned to pre-exposure levels. The manipulations involved in the tearing measurement technique (strip placement in the eye, tear collection for fifteen seconds, strip removal and placement in the

weighing chamber) required approximately thirty seconds, allowing, at most, two measurements per minute.

Dose vs. Concentration Effect

For this study, each subject participated in two exposures. Following pre-exposure tearing measurement, the right eye was exposed for five minutes to 50ppm. sulphur dioxide, with tearing measured in the right eye immediately after exposure. After tearing returned to pre-exposure levels, both eyes were simultaneously exposed for five minutes to 50ppm. sulphur dioxide, with tearing measured in the right eye immediately after exposure.

Results

Definition of the Contralateral Eye Tearing Response to Sulphur Dioxide

The results of this study are presented in Table 4.1.

The contralateral, unstimulated eye showed a marked tearing response with stimulation of the opposite eye. The mean percentage increase in contralateral eye tearing (116% ± 36%) was not meaningfully different from that of the exposed eye (130% ± 51%); the individual differences in the percentage increase in tearing between the contralateral, unstimulated eye and the exposed eye were inconsistent in direction.

Only one subject (R.H.) reported the subjective sensation of ocular irritation with sulphur dioxide exposure, and this subject only described irritation in the exposed eye. No subjects noticed the subjective sensation of tearing in the exposed or contralateral eye.

Definition of the Tearing Irritant Response Pattern

The results of this study are presented in Table 4.2, and the mean values of "excess tearing" (milligrams of difference between pre- and post-exposure tearing levels) are plotted in Fig. 4.1.

In this study it was only possible to maintain a

Table 4.1

Definition of the Contralateral Eye Tearing Response

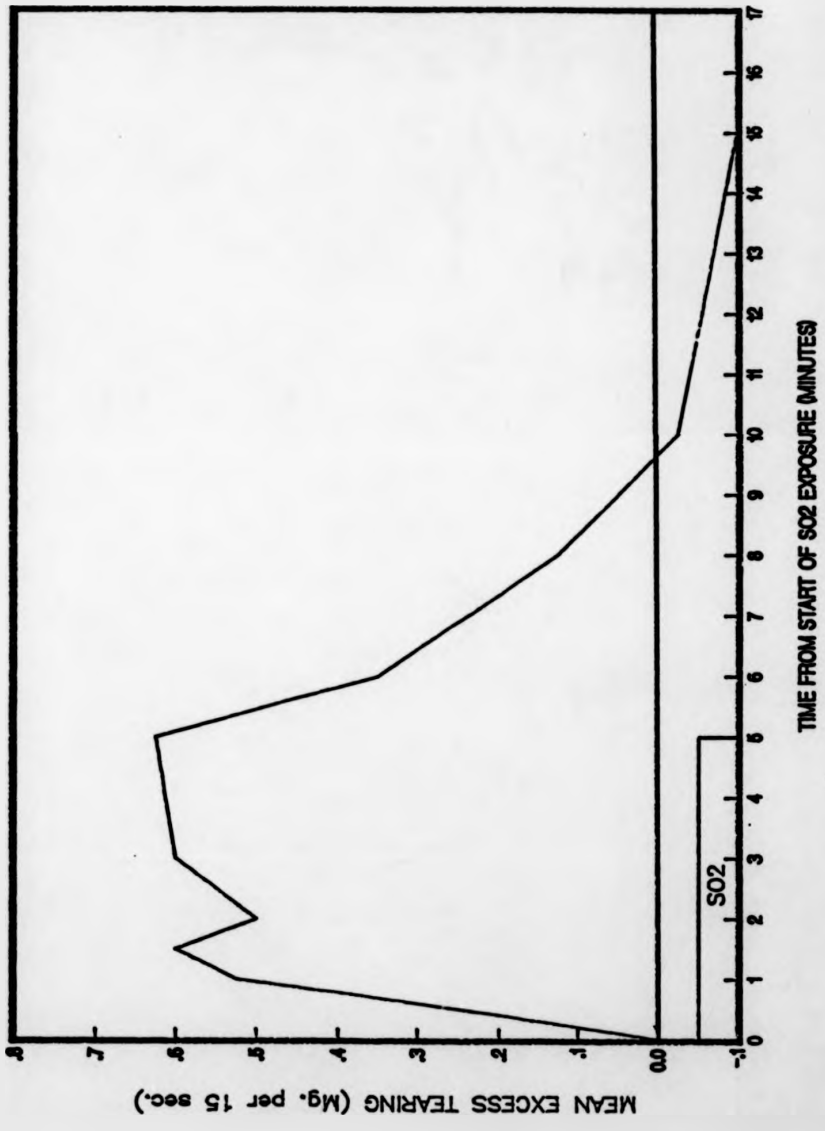
	Stimulated Eye			Unstimulated Eye		
Subjects	Pre-Expos.	Post-Expos.	% Change	Pre-Expos.	Post-Expos.	% Change
1. J.C.	0.4	1.1	175	0.5	1.2	140
2. B.G.	0.7	1.4	100	0.7	1.6	130
3. R.H.	0.3	0.9	200	0.3	0.8	167
4. S.A.	0.4	0.7	75	0.3	0.6	100
5. J.G.	0.6	1.1	85	0.7	1.2	70
6. R.S.	0.7	1.7	145	0.8	1.5	88
		Mean	130%			116%
		S.D.	51.2%			36.1%

Table 4.2

Irritant Tearing Response Pattern

Time From Start of Exposure (minutes)	Tears Collected (mg./15 sec.) Subjects 1. J.C.	2. R.D.	3. B.G.	4. R.H.	5. M.T.
0	0.4	0.4	0.7	0.3	0.8
1/2	0.6	0.7	1.1	0.5	1.0
1	0.9	0.9	1.4	0.8	1.2
1 1/2	0.8	1.0	1.4	0.9	1.5
2	0.7	0.8	1.5	0.9	1.2
3	0.9	1.1	1.5	0.8	1.3
5	0.8	1.1	1.4	0.9	1.5
End Exposure					
6	0.7	0.8	1.0	0.7	1.2
8	0.5	0.6	0.7	0.6	0.8
10	0.4	0.6	0.5	0.6	0.4
15	0.3	0.5	0.6	0.5	0.6

FIG. 4.1: TEARING IRRITANT RESPONSE PATTERN



rapid rate of measurement (one measurement every thirty seconds) for the first two minutes--during the rising portion of the response curve. Continued rapid measurements after this proved unfeasible because of the time required for readying and pre-weighing the measuring strips, and also because several subjects (R.D., R.H., and M.T.) reported a sensation of irritation at the measuring site (although no unusual tearing pattern was found).

In this study two subjects (R.D., B.G.) also reported the sensation of mild ocular irritation during the exposure period; none of the subjects noted the sensation of tearing with the irritant exposure.

Dose vs. Concentration Effect

The results of the "single" and "double" eye exposures are presented in Table 4.3.

In this study, no meaningful difference was found between the individual or mean percentage increases in tearing recorded in the right eye following exposure of one or both eyes.

The subjective sensation of ocular irritation was reported by one subject (M.T.) during the exposure period; this subject, however, noted no difference in the sensation of irritation between single and double eye exposures.

Table 4.3

Dose vs. Concentration Effect

	One Eye Exposed			Both Eyes Exposed		
Subjects	Pre-Expos.	Post-Expos.	% Change	Pre-Expos.	Post-Expos	% Change
1. J.C.	0.4	1.0	150	0.7	1.5	115
2. B.G.	0.5	0.9	80	0.4	0.8	100
3. M.T.	0.7	1.5	115	0.5	0.9	80
4. C.H.	0.3	1.0	233	0.5	1.4	180
5. S.A.	0.6	1.1	83	0.6	1.3	117
6. R.S.	0.6	1.5	150	0.4	0.8	100
		Mean	135%			115%
		S.D.	56.9%			34.4%

Discussion

The tearing response to sulphur dioxide shown in Fig. 4.1 is characterized by a rapid tearing increase with irritant exposure, a relatively stable plateau during the bulk of the exposure period, and a rapid (within fifteen minutes) return to approximate pre-exposure levels following exposure termination. This pattern is commonly seen in irritant responses. For example, as reviewed by Alarie (1973), similar irritant response patterns include: 1. the change in mouse respiratory rate with o-chlorobenzylidine malononitrile (CBMN) or low concentrations of capsaicin, and respiratory rate in guinea pigs with 2-nitrobutene, acrolein, and sulphur dioxide; 2. human subjective reports of upper respiratory tract and eye irritation with CBMN; 3. increases in human and guinea pig airflow resistance with sulphur dioxide; 4. increases in rabbit, dog, and guinea pig respiratory rate with phosgene, ozone, and nitrogen dioxide.

Perhaps the major difference between the human tearing response seen in the present study and those mentioned above is in the rate of response resolution after irritation. In human pulmonary studies, for example, the increase in pulmonary flow resistance caused by single exposures to 10-20ppm. sulphur dioxide frequently lasted for thirty minutes or more (Frank

1964), while the tearing response always terminated in less than fifteen minutes. The reason for this difference in response resolution in the eye and lung is not known, but may relate to differences in the nature of the "protective" response at these sites.

As described by Frank (1964), the respiratory response to sulphur dioxide is biphasic: an initial sensation of "irritation" or "burning" in the throat or chest, occasionally associated with coughing, is followed by a more prolonged period of increased airflow resistance. The change in pulmonary resistance has been related to bronchoconstriction rather than airway edema, and is stimulated both reflexly, through the vagus nerve, and locally, by direct action of the irritant gas on the bronchial smooth musculature (Salem and Aviado 1961, Widdicombe 1963).

Both phases of this pulmonary response can be characterized as "protective", because coughing and bronchoconstriction prevent the irritants from reaching the vital lower respiratory structures. Once in the lower respiratory tract, however, the irritant gases adsorb onto the bronchial walls. By decreasing air flow, bronchoconstriction hampers irritant clearance and, as a consequence, actually prolongs the irritant exposure. Several other pulmonary mechanisms also are known to be present that would slow the resolution of

the bronchial irritant response. Frank (1964), for example, has also shown that sulphur dioxide is released from the airways during exhalation--in effect "re-exposing" the lungs with each breath until the irritant is eventually washed out of the respiratory tract. Finally, Nadel and Tierney (1972) have found that bronchoconstriction, itself, is self-perpetuating--that it acts to prolong itself.

In contrast, the ocular tearing response is uncomplicatedly "protective". Tearing simply and effectively limits ocular irritant exposure by diluting and buffering irritants and by physically preventing their adherence or accumulation on the eye's surface. And, just as tearing quickly clears the irritant from the eye, the tearing irritant response would be expected to terminate promptly.

The actual speed with which the ocular irritant response terminates has been strikingly demonstrated in experimental studies on the cat (Lele and Weddell 1959). Here it has been shown that neural activity in the corneal sensory nerves stops virtually immediately after the end of mechanical or thermal corneal irritation. Because the corneal unmyelinated nerves carrying chemosensory information must be similar to those associated with heat or touch, it is likely that the chemical irritant response would show a similar "rapid

switching" electrical pattern. Of course, full neurological understanding of the eye's irritant response pattern will have to wait for the application of such single-neuron recording techniques to experimental chemical corneal irritation, but the rapid response of corneal sensory nerves seems well reflected in the rapidity of onset and termination of the tearing irritant response.

The similarity of the initial rising phase of the irritation response curve for different organs and different species also suggests possible anatomical similarities in the various irritant response systems. For example, in the upper and lower respiratory tract specific irritant receptors have been identified (Widdicombe 1963, Canua 1969, Sellick and Widdicombe 1970, Paintal 1973, Widdicombe 1977, Das et al 1978, 1979), consisting of small, unmyelinated axons with no specific end-organ structures. As described in Chapter 2, the trigeminal nerve fibers that penetrate the corneal epithelium have much the same appearance and this, coupled with the similarity in the onset phase of the irritant response curve, further suggests that these corneal trigeminal fibers are themselves (or are associated with) the ocular chemoreceptors.

Irritant Response Curves

Anatomical similarity of the lung and eye chemoreceptors is also suggested by the similarities in these organs' irritant response thresholds. The ocular tearing thresholds of approximately 5ppm. sulphur dioxide and 55ppm. ammonia found in the present study are close to the bronchoconstriction thresholds of 1-2ppm. sulphur dioxide (Frank 1966, Douglas 1974) and approximately 85ppm. ammonia (Douglas 1974). Because the thresholds for bronchoconstriction were determined by plethysmography--a technique generally acknowledged to be of great sensitivity--the similarity of the thresholds arrived at by the relatively simple tearing measurement technique is all the more striking.

As can be seen in Tables 3.1 through 3.6, the individual ocular responses to each irritant gas and gas concentration showed a considerable range of variation. This individual variability has long been recognized as a characteristic of the human irritant response and has previously been discussed with reference to the eye by Doyle et al (1961), and to the lung by Sim and Pattle (1957), Frank (1964), Douglas (1975, 1980) and Alarie (1973). The anatomical and/or chemical basis for this variability is not known, but it has been found to be unrelated either to the subject's immune status (Douglas 1975) or prior participation in

irritant exposure studies (Doyle et al 1961). In the present study, in which environmental factors (drafts, room temperature and lighting) were carefully controlled, the persistence of a wide range of individual responses suggests that the variability is due to internal rather than external factors.

One possible cause for the wide range of responses might be the process of adaptation. For many irritants (ammonia, in particular) adaptation can be quite rapid, producing major alterations in the magnitude of the irritant response (Ferguson et al 1977, Verbeck 1977). In the present study, this may have been an important factor in the relatively small responses of three subjects (P.B., W.C., and B.B.) to 250ppm. ammonia. These subjects had worked for more than one year in the chemical byproducts area of a manufacturing plant where they were regularly exposed to low levels of ammonia. From the report of Ferguson et al (1977), it is known that such exposures, over as brief a period as two weeks, can all but eliminate the subjective responses to 200ppm. ammonia. Although in these subjects prior exposure is known, it is also possible that other subjects' irritant responses might have been influenced, to an unpredictable degree, by home, laboratory, or work exposures to the common irritant gases studied, or even possibly by the five minute

experimental exposures themselves.

The use of only three concentrations to define the response "curve", of course, was less than ideal. However, the approximately 0.1-0.2mg. range of unstimulated tearing (found in the "sham" experiments, see Tables 3.9 to 3.11, and in exposures to irritant concentrations below the response threshold) meant that the tearing measurment technique would probably not be useful at either low irritant concentrations or to define small portions of the response curve between two close irritant concentrations. This methodological problem, combined with the restriction placed on the maximum irritant concentration, means that the shapes of both extremes of the response curves are necessarially somewhat speculative.

Pulmonary response studies using sulphur dioxide and ammonia have shown that the response vs. log dose relationship tends to be linear towards the middle of the "physiological" concentration range, becoming somewhat unpredictable at the extremes (Frank 1964, Schuck et al 1966, Alarie 1973). Because the maximum and middle sulphur dioxide and ammonia concentrations used in this study were only moderate but still provoked robust increases in tearing (well above the "noise" of the measuring method), it is likely that the slopes of the mid-portions of the current study's response curves

are reliable.

Measurement of the tearing responses to sulphur dioxide and ammonia concentrations below 2pp. (SO_2) and 10ppm. (NH_3) would be of great interest. Improved tearing measurement techniques would be necessary to define the response to these lower concentrations, but this should definitely be studied in future investigations. For example, definition of the occasionally eccentric initial portion of the response curve would have allowed the important question of whether the irritant response was "all or none" or "proportional"--as suggested by the Weber-Fechner "law" or other proportional response principles (Stevens 1970)--to be answered.

Based on a comparison of the objectively measured threshold for bronchoconstriction and the subjective threshold for eye irritation, Douglas (1975) concluded that the lung was more sensitive to irritants than the eye. He recognized, of course, that these thresholds were not strictly comparable, and that objective ocular response data was needed. The present study's objectively determined sulphur dioxide (5ppm.) and ammonia (55ppm.) tearing thresholds were considerably lower than the subjective ocular irritation thresholds found by Douglas (168ppm. sulphur dioxide and 110ppm. ammonia). In fact, the objective tearing

threshold for sulphur dioxide was only slightly higher than this gas' bronchoconstriction threshold (1ppm.), and the tearing theshold for ammonia was actually below the ammonia bronchoconstriction threshold of 85ppm. or more (Douglas 1975, Verbeck 1977). Thus, in view of the new objective ocular data, it appears that the irritant gas sensitivity of the eye and the lung must be considered approximately equal.

Histologically, this eye-lung parity is somewhat surprising. A recent study found the unmyelinated nerve ending density in the corneal epithelium to be the highest in the human body, approximately three to six hundred times greater than that in the skin and twenty to forty times greater than that in the mouth and respiratory tract (Fozsa and Beuerman 1982). In view of this extreme density of corneal free nerve endings, it might have been expected that the eye would actually be even more irritant-sensitive than the lung. The findings of the present study, however, do not support this speculation.

If other studies confirm that the eye and lung are equally sensitive to irritants, it would seem that the density of unmyelinated nerve endings is not the critical factor in determining an organ's chemical irritant sensitivity--as opposed, for example, to this parameter's importance for touch or two-point discrimi-

nation. This observation is important for understanding the ocular chemoreception process, because it suggests that irritant "perception" is a complex function of the individual receptor rather than the simple summed product of the number of receptors activated per unit of exposed surface. The "intensity" of the afferent signal arriving at the central nervous system would then be determined by a graduated response of individual chemoreceptors rather than a simple "all or none" receptor response. This speculation is also supported by animal studies showing that individual corneal nerve endings are able to respond to non-chemical stimuli in just such a proportional manner (Tanaka 1973, Mark and Maurice 1977).

The large differences between the objective ocular thresholds and the subjective thresholds found by Douglas were predictable. Similar objective-subjective differences have been reported for bronchoconstriction (Frank et al 1962, Alarie 1973)--the report of Frank et al concluding that "subjective responses were not reliable indexes of the degree of change in respiratory mechanics". Summarizing various published and unpublished studies of acute ocular exposures to sulphur dioxide, Grant (1974) concluded that slight subjective eye irritation can occasionally be found at 10ppm., with consistent irritation only appearing above

50ppm. These levels are lower than those found in the present study and Douglas' 1975 study but, again, are still well above sulphur dioxide's objective tearing threshold of 5ppm. Given this subjective-objective threshold difference, Frank et al's comments would seem to apply equally well to the eye.

The reason for the subjective-objective ocular threshold differences, and for the relatively high subjective thresholds found in the present study, is unknown. The subjects in this study were asked to report any sensation of eye discomfort experienced during the exposure period. However, unlike some subjective ocular exposure studies (Renzetti and Schuck 1961, Schuck et al 1966), no specific irritancy scaling techniques were used. The use of such scales might have increased the subjects' recall of slight degrees of irritation that would not otherwise have been reported using the more informal techniques of the present study and that of Douglas (1975). Of course, it is also possible that the gap between subjective and objective thresholds is simply another aspect of the irritant response's protective function--working essentially "unconsciously" at low irritant levels to protect the vital structures of the eye and lung.

Contralateral Eye Response

The contralateral eye tearing response was quite striking, being prompt and, in so far as the technique was able to detect, similar in magnitude to the response of the directly exposed eye. This irritant-induced contralateral tearing response apparently has not been previously studied. Investigations of the inflammatory response to corneal nitrogen mustard application have shown a more variable contralateral effect (inflammation developing in only one-quarter to one-half of the contralateral eyes), quite different from the consistent pattern seen in the present study (Akagi et al 1955, Davson 1962, Moses and Holenkamp 1971, Chiang and Thomas 1972, Maul and Sears 1976a,b, Kottow and Seligman 1978, Jampol and North 1979).

Little is known about the mechanism of the contralateral tearing effect, but its rapidity of onset suggests a neural rather than a chemical basis. The possibility of a neural mechanism is also supported by studies in which nerve blocking techniques--contralateral trigeminal section or retrobulbar lidocaine injection (Maul and Sears 1976a,b, Jampol and North 1979)--were shown to interfere with the contralateral inflammatory response. A neural mechanism is also suggested by blink reflex studies in which stimulation of one eye by light corneal touch or a flash of light led

to contralateral blinking with a latent period of only six milliseconds--a delay so brief that it could only be explained by a neural mechanism (Hiraoka and Shimamura 1978, Mikano et al 1983).

Dose vs. Concentration Effect

Taking advantage of the contralateral tearing response, the present study made a preliminary determination as to whether the ocular response to sulphur dioxide was dose- or concentration-dependent. From sessions in which first one and then both eyes were exposed, it was found that tearing varied only with sulphur dioxide concentration: the tearing response from simultaneous exposure of both eyes to 50ppm. sulphur dioxide (thus doubling the total dose as compared to the single eye exposure) produced a response similar in magnitude to that found with exposure of only one eye.

This finding agrees with the work of Douglas (1975) and Alarie (1977) on the lung and eye. Because the primary focus of the present study was on method development and descriptive physiology of the tearing irritant response, the ocular responses to a large group of irritants with potentially different response characteristics were not determined. However, from the work of Douglas (1975) it is likely that irritants like

the aldehydes, whose subjective ocular responses are dose-related, would also have dose-related objective responses. Expanding the scope of this study to include dose-dependent irritants like formaldehyde and acrolein would be of great interest, and a sample protocol including this type of investigation is given in the Appendix.

CHAPTER 5

DEFINITION OF THE AFFERENT PATHWAY FOR THE HUMAN OCULAR IRRITANT RESPONSE

Introduction

In the studies described in this Chapter, a number of physical and chemical blocking techniques were used to define the afferent pathway for the tearing irritant response. The blocking agents studied included impermeable corneal contact lenses, topical anesthetics, beta-adrenergic antagonists, prostaglandin synthesis inhibitors, and section of the spinal tract of the trigeminal nerve (in subjects who had undergone this procedure as a treatment for trigeminal neuralgia). In addition, the neural pathway was also investigated by measuring vagally-mediated changes in heart rate in response to sulphur dioxide eye irritation.

Contact Lenses

Impermeable corneal contact lenses were used to block the irritant tearing response by physically "shielding" the central portion of the cornea. This

approach was attractive for both practical and theoretical reasons. First, in the population from which the subjects for this study were drawn the use of contact lenses was common, so volunteer subjects well-acclimated to contact lens wear were easily obtained.

Secondly, the interaction between contact lenses and the eye has already been the subject of considerable investigation. Modified contact lenses have been used in <u>in vivo</u> studies of human corneal metabolism (Hill and Fatt 1964, Jauregui and Fatt 1971), and the shielding effect of contact lenses has been employed clinically to protect portions of the eye following operation or injury. A number of animal studies have found that impermeable contact lenses can protect the cornea from liquid chemical splashes and solvent fumes (Guthrie and Seitz 1975, Nilsson and Andersson 1982), further supporting speculation that the lenses may also shield the corneas of human subjects from irritant gases.

Finally, the use of impermeable contact lenses to shield the cornea is supported by studies of the physics and chemistry of the contact lens material (polymethylmethacrylate) itself. Here it has been shown that such lenses are, in fact, completely impermeable to atmospheric gases (Fatt 1972). This finding has also been confirmed physiologically by

studies showing that the oxygen tension under a tight-fitting impermeable contact lens approaches zero within two minutes, again demonstrating that the lens can prevent atmospheric gases from reaching the cornea (Hill and Fatt 1964, Jauregui and Fatt 1971).

With a normal-fitting impermeable contact lens, the metabolic needs of the cornea (itself avascular) are supplied by oxygen dissolved in tears and diffusing out of the aqueous humor and limbal capillaries (Smelser 1952). Elegant studies have shown that each blink "rocks" the contact lens, permitting an inflow of tears from the unshielded areas of the eye. This slight tear exchange is sufficient to maintain an oxygen tension of approximately 15 to 20mmHg at the corneal surface (Fatt and Hill 1970). The large difference between the oxygen tension in tears freely exposed to room air (155mmHg) and that under normally-fitting contact lenses (20mmHg) also demonstrates that impermeable contact lenses have a significant shielding effect under ordinary wearing conditions (as would be expected in our volunteer subjects).

For the present study, it was important that acclimation to contact lenses did not alter normal ocular responses so that the results from these subjects could be meaningfully compared to those from non-contact lens-wearers. Unfortunately, the ophthalmo-

logical literature is equivocal on the question of the "normalicy" of contact lens-wearers' eyes. For example, it has been found that a corneal surface oxygen tension of 10-20mmHg is just sufficient to prevent corneal thickening and other pathological corneal changes (Polse and Mandell 1970). The fact that these oxygen levels can be achieved with impermeable contact lenses (Hill and Fatt 1964) implies that contact lens-wearers' corneas are intact, and that corneal pathology would not exist to interfere with these subjects' irritant responses. This view is also supported by Farris et al's 1981 tearing study, in which no significant differences were found between contact lens-wearers and non-contact lens-wearing subjects in basal tear volume, reflex tear flow, and various tear constituents.

Other studies, however, have shown significant corneal changes after normally-fitting contact lenses have been worn for as little as several hours (Mandell and Harris 1968, Farris et al 1971, Millodot 1975). Most characteristic is corneal thickening, a change that has been related to chronic corneal oxygen deprivation, epithelial irritation, lactate accumulation, and over-hydration (Hedbys and Mishima 1962, Klyce 1981). After removal of the lenses, the corneal thickening resolves over thirty minutes to several hours (Farris et al 1971, Ko 1973, Millodot 1975). A number

of studies have also reported short- and long-term decreases in corneal sensitivity with contact lens wear (Boberg-Ans 1955, Morganroth and Richman 1969, Knoll and Williams 1970, Ong 1972, Ko 1973, Millodot 1975, 1976, 1977, Draeger et al 1980, Tanelian and Beuerman 1980, Zeithoun 1980). In some of these studies the changes in corneal sensitivity have been related to corneal edema, but this could only explain the short-term decreases in sensitivity because any edema present fully resolves within hours. The reason for the more prolonged sensitivity decrease--occasionally persisting over several years--remains unknown (Millodot 1977). Because these studies suggested the possibility of chronic changes in the eyes of contact lens-wearing subjects, particular caution was taken in experimental design to insure that the shielding effect of the contact lenses could be separated from any underlying changes in ocular anatomy or physiology peculiar to contact lens-wearers.

Topical Anesthetics

In this study topical anesthetics were used to block the ocular irritant response by locally inhibiting corneal nerve action potentials. Because of the lipophilic residues in their chemical structure, topical anesthetics quickly bind to and penetrate mem-

branes, gaining access to superficial nerve endings and the distal portions of terminal axons (Ito et al 1964, Blaustein and Goldman 1966, Seeman 1972). It is now generally accepted that the primary site of action of these agents is the cell membrane, and that they do not affect (or have only secondary affects on) intracellular processes (Blaustein and Goldman 1966, Ritchie 1975). Careful studies of neuron oxygen consumption, for example, have shown that nerve block can be accomplished at anesthetic doses much lower than those required to inhibit axonal metabolism, although increasing doses of anesthetics will ultimately exert more far-reaching pharmacological effects, depressing intracellular chemical reactions (Carribbee et al 1952, Carribbee and Posternak 1952, Augsburger and Hill 1972). The exact manner in which topical anesthetics are able to reversibly affect axon membranes is not known with certainty, but the most likely sites of action are the membrane enzyme systems regulating ion transport (Taylor 1959, Nathan and Sears 1961, 1962, Seeman 1972).

Of particular interest for the present study is that neuron size has been found to be an important criteria in determining anesthetic effectiveness, with topical anesthetics blocking small axons (such as those in the corneal epithelium) much more rapidly than

larger axons (Franz and Perry 1974). These studies also have shown that exposure of a relatively small area of the axon to a topical anesthetic (the nerve ending, for example) blocks that area more completely than adjoining, more proximal portions of the axon (Franz and Perry 1974).

Although cocaine was used as a blocking agent in an early, qualitative study of human ocular irritation (Mutch 1944), it was not considered for the present study because of its toxicity and unpredictability--sometimes altering the corneal epithelium (Augsburger and Hill 1972, Brewitt et al 1980) and occasionally precipitating an ocular inflammatory reaction (Snow 1972). Instead, the topical anesthetic 0.4% benoxinate was employed. This agent is an easily controlled procaine derivative with a rapid onset (within one minute), a relatively short duration of action (less than thirty minutes), and only minimal effects on the corneal epithelium (Schlegel and Swan 1954, Linn and Vey 1954, Augsburger and Hill 1972, Snow 1972). Although all topical anesthetics are somewhat irritating (producing a "burning" sensation with instillation), the dose of benoxinate required for effective anesthesia is low (Polse et al 1978) and the irritant effect rapidly subsides (Snow 1972).

Beta-Adrenergic Antagonists

A potent beta-adrenergic antagonist, timolol, was used to determine whether corneal beta-adrenergic receptors played a role in the ocular irritant response (Neufeld and Page 1977, Neufeld et al 1978, Wiederholt et al 1983). Single drop applications of timolol rapidly produce effective ocular beta-blockade (Neufeld et al 1983). From studies using H^3-timolol, it has been shown that this agent quickly penetrates the cornea and aqueous humor; it then binds to the pigmented tissues of the iris and ciliary bodies, from which it is slowly released over the next twelve to eighteen hours (Schmitt et al 1980, Araie et al 1982, Salmen et al 1984).

Clinically, timolol lowers intraocular pressure in both glaucoma patients (Zimmerman and Kaufman 1977) and normal volunteers (Katz et al 1976). However, the relationship between beta-adrenergic blockade and decreased intraocular pressure remains unknown. A recent study has found that timolol's effect is unrelated to prostaglandins (Araie and Takase 1983); other studies have suggested that it decreases ciliary body dopamine levels (Wantanabe and Chiou 1983), a change that in some unknown way decreases the blood flow to the iris root, ciliary body, and choroid, diminishing aqueous humor formation (Zimmerman et al 1977, Coates and

Brubaker 1978, Sontag et al 1978, Neufeld et al 1983, Lehto et al 1984).

Ocular side effects have been reported with timolol use, but generally have been slight and are directly proportional to dose and duration of treatment (Zimmerman et al 1983). For the present study, the side effect of greatest concern was the sensation of ocular irritation with initial use of the drops--although this symptom was noted by fewer than ten percent of all individuals treated (Zimmerman et al 1983).

Also of concern were reports that timolol can cause both short- and long-term decreases in corneal sensitivity (van Buskirk 1979, Calissendorf 1981, Draeger et al 1983). Most beta-adrenergic antagonists are known to have an anesthetic effect (Kitazawa and Tsuchuska 1980), but careful study in both animals and humans has generally shown no significant long-term changes in corneal sensitivity or tear production (Katz 1978, Kitazawa and Tsuchusaka 1980). Using an extremely precise electronic measurement of corneal sensitivity, Draeger et al (1983) did report a slight anesthetic effect with timolol, but this lasted for no more than three minutes. Because the transient anesthesia was dose-related, the minimal effective dose (one drop of 0.25% timolol solution) was selected for

the present study.

Prostaglandin Synthesis Inhibitors

The role of prostaglandins in the pathogenesis of the ocular inflammatory response was briefly reviewed in Chapter 2. Evidence in support of the relationship between prostaglandins and inflammation came from both direct measurement of increases in prostaglandin (particularly PGE_2) activity in the anterior chamber after corneal application of nitrogen mustard, and from studies in which the ocular inflammatory response was blocked by pre-treatment with prostaglandin synthesis inhibitors.

The most widely studied prostaglandin synthesis inhibitor is indomethacin. It is now well established that indomethacin inhibits ocular prostaglandin synthetase (Neufeld et al 1972), and is effective either systemically (Jaffe et al 1973, Podos et al 1973, Conquet et al 1975, Unger et al 1977, Klein et al 1979, Camras and Bito 1980a,b, Algvere and Hohnsson 1981, Baikoff et al 1981, Kremer et al 1982) or topically (Miyake 1980, Spinelli and Krohn 1980, Kulkarni and Srinivasan 1981, Kulkarni et al 1981, van Haeringen and Glasius 1981, Yanuzzi et al 1981, Sanders et al 1982, van Haeringen et al 1982, Green et al 1983, Keulen de Vos et al 1983, Sanders et al 1983, Urner-Block 1983,

Williams et al 1983)--although the topical route is somewhat more effective (Sanders et al 1983) and avoids the possiblity of systemic side effects. In these and other studies, indomethacin was found to have no direct ill effects on the cornea (Palimeris et al 1972, Srinivasan 1981), and to be effective in supressing prostaglandin synthesis in both superficial ocular tissues (conjunctiva) and deeper in the anterior chamber (Kulkarni and Srinivasan 1981).

For the present study, the role of prostaglandins in the ocular irritant response was determined by measuring the tearing provoked by sulphur dioxide after pre-treatment with 200mg. (50mg. QID) of oral indomethacin. The systemic route of administration was chosen because a commercial topical indomethacin preparation had not been approved for general medical use at the time of this study.

In the reports cited above, the effectiveness of systemic indomethacin in preventing ocular PGE_2 synthesis was found to be dose-related: 100mg. (25mg. QID) or more of oral indomethacin, beginning twenty-four hours prior to ocular surgery, could supress post-operative inflammatory changes (Klein et al 1979, Baikoff et al 1981), but 75mg. (25mg. TID) or less was often ineffective. Because the serum half-life of indomethacin is relatively long (twenty-four hours) and

the present study's pre-treatment period was to be only one day (minimizing the possibility of systemic side effects), a relatively large indomethacin dose (200mg.) was selected. In view of the dose-related effect of indomethacin, it was believed that at this dose ocular prostaglandin synthetase activity would definitely be inhibited.

Trigeminal Tractotomy

In order to determine the site of central nervous system integration of the ocular chemosensory afferents, sulphur dioxide-induced tearing was studied in subjects who had undergone section of the trigeminal spinal tract for relief of trigeminal neuralgia (tic doloureux). Individuals with this condition experience paroxysms of severe pain in one or more of the trigeminal divisions. The cause of trigeminal neuralgia is not known, but it is often hereditary (increased incidence in descendents of those afflicted, more common in women than men, more common on the right than the left side), may be associated with traction or compression of a portion of the trigeminal nerve by a normal anatomical structure or benign tumor, and is not generally associated with other underlying neurological abnormalities (Janetta 1977, Voorhies and Patterson 1981).

Although known since antiquity (Dandy, 1929, for

example, cites Avicenna, c.1000 A.D., for the first description of this condition) trigeminal neuralgia was believed to be an abnormality of the facial nerve until the studies of Magendie and Bell in the early nineteenth century showed the trigeminal nerve to be responsible for facial sensory function. Once this fundamental distinction had been made, pain relief was achieved by Lizars (1821) and others through section of the appropriate peripheral trigeminal branch. Unfortunately, this simple procedure often led to severe trophic changes distal to the section site and, for much of the next century, neurosurgeons sought new approaches to relieve trigeminal pain while sparing other trigeminally-mediated functions (facial and ocular touch, in particular).

The effort to improve the surgical treatment for trigeminal neuralgia was aided by careful anatomical studies of fibers carrying specific types of sensory information within the trigeminal nerve. By the early 1930's, through the work of Horsley, Dandy, Spiller, Frazier, Grant, and others, it was found that pain and touch were separated in the trigeminal nerve: in the posterior fossa, the fibers carrying light touch and, possibly the corneal reflex (the "accessory fibers") run medial to the main sensory trunk (Gerard 1923, Dandy 1929, Illingworth 1974) and, within the cranium

itself, the trigeminal sensory afferents organize into two distinct tracts--the descending or "spinal" tract, carrying fibers for pain and temperature sensation to the spinal trigeminal nucleus in the upper cervical region, and the ascending or "main sensory" tract, carrying touch and proprioception to the main sensory trigeminal nucleus (Walker 1934).

The recognition of this separation of pain and touch in the trigeminal system made possible new surgical procedures for the treatment of trigeminal neuralgia. Among the most promising was section of the spinal trigeminal tract, first suggested by Ranson (1931) and independently demonstrated by Sjoqvist (1938) and Dogliotti (1938). On the whole, the tractotomy procedure was a significant theraputic advance, but several problems soon became apparent (Grant 1955, Denny-Brown and Yanigasawa 1973). These included technical difficulties in the control of bleeding in the posterior fossa, difficulty in determining the extent of tract section during surgery, and a small but worrisome number of patients in whom facial anesthesia was achieved while the neuralgia either remained or recurred (Rowbotham 1938, Olivecrona 1942, Grant 1955)--presumably because fibers were occasionally mixed within the tracts (Weinburger and Grant 1942). In the 1960's, when improved medical treatment and effective

but less invasive surgical procedures became available, the tractotomy procedure fell into disuse.

The precise number of patients who underwent tractotomy is unknown but some of these patients are still being followed at centers where the procedure was commonly performed. Although corneal touch remained intact and trophic changes were not common, most patients had been warned to take particular care to protect their anesthetic eye. As a consequence, none who were approached would agree to the sulphur dioxide exposures used in the other phases of this study. However, a modified exposure procedure was devised using freshly crushed onion as the ocular irritant--a technique derived from early studies of the tearing process (Thompson and Galliardo 1936, 1941, Brunish 1956, 1957)--and a subjective, non-invasive assessment of tearing. This revised procedure was acceptable to three individuals in whom the post-tractotomy tearing irritant response was studied.

Trigeminal-Vagal Interaction

That the trigeminal nerve is the afferent pathway for corneal sensation has already been well established. In the present study, an attempt was made to demonstrate the presence of this pathway in human beings by using known trigeminal-vagal interactions

(the oculo-cardiac reflex) in studying the effect of sulphur dioxide eye irritation on heart rate.

Subjects and Methods

Subjects

Eighteen volunteer subjects (13 males and 5 females) participated in these studies. The characteristics of the subjects and the criteria for their selection were as described in Chapter 3.

Methods

As noted above, six separate studies were carried out to define the afferent pathway for the ocular tearing irritant response:

1. Determination of the effect of impermeable corneal contact lenses on the irritant response.

2. Determination of the effect of topical anesthesia with 0.4% benoxinate on the irritant response.

3. Determination of the effect of ocular beta-adrenergic blockade with 0.25% timolol on the irritant response.

4. Determination of the effect of prostaglandin synthesis inhibition by systemic indomethacin on the irritant response.

5. Determination of the effect of section of the trigeminal spinal tract on the irritant response.

6. Determination of the effect of chemical ocular irritation on heart rate.

The irritant gas used in all these studies was

50ppm. sulphur dioxide. Irritant gas preparation and tearing measurement were as described in Chapter 3. Specific details of the exposure procedures are given below.

The Effect of Contact Lenses on the Ocular Irritant Response

All subjects in this study had worn contact lenses for more than five years (mean twelve years). Subjects removed one contact lens one hour before irritant exposure. Following this acclimation period, pre-exposure tearing levels were measured in the eye in which the contact lens remained (the "shielded" eye). A nose clamp was then applied, the unshielded eye was exposed to 50ppm. sulphur dioxide for five minutes, and tearing was measured from the shielded eye immediately after exposure and at fifteen minutes post-exposure. After another thirty minute interval, tearing levels were again measured in the shielded eye. The shielded eye was then exposed to 50ppm. sulphur dioxide for five minutes. Tearing was now measured in the shielded eye immediately and at fifteen minutes post-exposure.

The Effect of Topical Anesthesia on the Irritant Response

In this study, one drop of 0.4% benoxinate was

instilled into the right eye following a pre-exposure tearing measurement. In order to determine whether benoxinate was an irritant, tearing measurements were then made at one and five minutes after benoxinate instillation. At ten minutes post-benoxinate, ocular anesthesia was tested by touching the caruncle, sclera, and corneal margin lightly with the tip of a strip of filter paper; the subject was asked to report any subjective sensations of touch and/or pain. If the eye was anesthetic, pre-sulphur dioxide exposure tearing measurements were made in the right eye. Using the split-eyepiece exposure system described in Chapter 4, the right eye was then exposed to 50ppm. sulphur dioxide for five minutes while the left eye was shielded. Tearing was measured from the right eye immediately after exposure and at five and fifteen minutes after exposure. Subjects were then requested to remain in the laboratory until the ocular anesthesia abated.

The Effect of Beta-Adrenergic Blockade on the Irritant Response

For this study, following pre-exposure tearing measurements and pulse rate determination, one drop of 0.25% timolol solution was instilled into each eye. Tearing measurements were repeated at one and five minutes post-timolol. If tearing remained at the

pre-timolol levels, both eyes were exposed to 50ppm. sulphur dioxide for five minutes. Tearing and heart rate were measured immediately after sulphur dioxide exposure and at five and fifteen minutes after exposure.

The Effect of Systemic Indomethacin on the Irritant Response

Subjects participating in this study took one 50mg. indomethacin capsule every six hours, beginning approximately twenty-six hours prior to the exposure session (the last dose was taken approximately two hours before the exposure). Following pre-exposure tearing measurements, both eyes were exposed to 50ppm. sulphur dioxide for five minutes. Tearing measurements were made immediately after exposure and at five and fifteen minutes after exposure.

The Effect of Trigeminal Spinal Tractotomy on the Irritant Response

Three subjects participated in this study. Their average age was seventy-one years and, consequently, they represent a very different population than the other volunteer subjects.

For this study, the eye was irritated by the vapour of freshly chopped onion held approximately three inches below the eye on the side on which tractotomy had been performed. The contralateral eye (the

"intact" side) was shielded by a single eyepiece of the exposure goggle set. Tearing was determined subjectively by observation of changes in the size of the lower meniscus.

The Effect of Chemical Ocular Irritation on Heart Rate

In this study, pre-exposure heart rate was measured by a recording electrocardiograph. Following this determination, both eyes were exposed to 50ppm. sulphur dioxide for five minutes. Heart rate was measured at one and five minutes after the start of exposure, and at five and fifteen minutes after exposure.

Results

The Effect of Contact Lenses on the Ocular Irritant Response

The results of this study are presented in Table 5.1.

In all subjects, exposure of the unshielded eye led to a significant ($p<0.01$) increase in tearing in the shielded eye. However, exposure of the shielded eye to sulphur dioxide produced no meaningful change in tearing in the shielded eye in any subject.

The tearing response evoked in the shielded eye by irritation of the unshielded eye was similar, in duration and percentage change in tearing (the mean increase was 94% ± 26%), to that previously found in non-contact lens wearing subjects. Because of this, the absence of a tearing response with exposure of the shielded eye to sulphur dioxide was believed to represent a true blocking effect on the afferent limb of the irritant reflex, rather than an artifact related in some other way to contact lens wear.

In this study, no subjects reported the subjective sensations of eye irritation or tearing.

The Effect of Topical Anesthesia on the Ocular Irritant Response

The results of this study are presented in

Table 5.1

The Effect of Impermeable Contact Lenses on the Tearing Irritant Response

Unshielded Eye

Subjects	Pre-Expos.	Post-Expos.	% Change	15' Post
1. J.C.	0.3	0.6	100	0.3
2. B.G.	0.8	1.3	62	0.7
3. S.A.	0.8	1.7	112	0.6
4. J.G.	0.6	1.4	133	0.7
5. W.C.	1.1	2.0	72	1.0
6. R.C.	0.6	1.1	83	0.6
			Mean 94%	
			S.D. 26%	

Shielded Eye

Subjects	Pre-Expos.	Post-Expos.	% Change	15' Post
1. J.C.	0.4	0.4	0	0.3
2. B.G.	0.7	0.7	0	0.6
3. S.A.	0.8	0.8	0	0.7
4. J.G.	0.5	0.4	-20	0.5
5. W.C.	0.9	1.0	11	1.0
6. R.C.	0.6	0.6	0	0.7
			Mean -2%	
			S.D. 10%	

Table 5.2.

In all subjects, 0.4% benoxinate instillation was associated with a small but significant ($p<0.05$) increase in tearing. The mean percentage increase in tearing at one minute post-benoxinate was 53% ± 18%--a response approximately equivalent to that produced by exposure to 10ppm. sulphur dioxide. The benoxinate-induced irritation resolved quickly and, in all cases, the tearing level had returned to pre-benoxinate levels by five minutes after instillation.

All of the subjects reported that benoxinate was irritating, being associated with a slightly painful, "burning" sensation. However, this discomfort rapidly subsided and, by the one minute post-benoxinate tearing measurement, all subjects reported only that the treated eye felt "numb".

Tested at ten minutes post-benoxinate, all subjects evidenced ocular anesthesia: they were not able to perceive the filter paper strip touching the caruncle or sclera, and reported no pain with touching of the corneal margin. No changes in conjunctival vasculature or gross tearing were observed. Repeat tearing measurements, made after the subjects' eyes were tested for anesthesia, showed no change from the previous levels.

Exposure of the anesthetic eye to sulphur

Table 5.2

The Effect of Topical Anesthesia on the Tearing Irritant Response

Benoxinate Instillation

Subjects	Pre-Expos	Post-Expos.	% Change	5' Post.
1. J.C.	0.7	1.0	43	0.6
2. R.D.	0.6	0.9	50	0.6
3. R.H.	0.6	1.0	66	0.5
4. C.H.	0.8	1.0	25	0.8
5. M.T.	0.4	0.7	75	0.5
6. P.O.A.	0.5	0.8	60	0.5
			Mean 53%	
			S.D. 18%	

Sulphur Dioxide Exposure

Subjects	Pre-Expos.	Post-Expos.	% Change	15' Post.
1. J.C.	0.6	1.7	183	0.5
2. R.D.	0.5	1.0	100	0.5
3. R.H.	0.5	1.2	140	0.6
4. C.H.	0.7	1.2	71	0.7
5. M.T.	0.4	0.9	125	0.5
6. P.O.A.	0.6	1.0	66	0.5
			Mean 114%	
			S.D. 45%	

dioxide produced a characteristic tearing response in all subjects. The mean percentage increase in tearing measured immediately after exposure was 114% ± 45%. The tearing response resolved rapidly after exposure and in all subjects tearing had returned to its pre-exposure levels by fifteen minutes post-exposure.

No subjects reported the subjective sensation of eye irritation in response to the sulphur dioxide exposure.

The Effect of Beta-Adrenergic Blockade on the Irritant Response

The results of this study are presented in Table 5.3.

None of the six subjects in this study reported the subjective sensation of eye irritation with instillation of 0.25% timolol solution, and timolol instillation was associated with no meaningful change in tearing when measured five minutes after use. Heart rate also was not meaningfully affected by ocular timolol instillation.

Exposure to sulphur dioxide produced a characteristic tearing response with a mean percentage increase of 126% ± 45%. This response resolved promptly after the exposure and tearing returned to pre-exposure levels within fifteen minutes. Heart rate measured at five and fifteen minutes post-exposure was found to be

Table 5.3

The Effect of Beta-Adrenergic Blockade on the Tearing Response (mg. tears/15 sec.)

Timolol Instillation

Subjects	Pre-Expos.	Post-Expos	% Change	5' Post
1. J.C.	0.5	0.5	0	0.4
2. R.D.	0.8	0.9	12	0.7
3. R.M.	0.3	0.3	0	0.3
4. P.B.	0.5	0.6	20	0.5
5. J.B.	0.6	0.5	-17	0.5
6. B.B.	0.6	0.6	0	0.7
7. R.A.	0.7	0.6	-14	0.6
8. J.D.	0.5	0.5	0	0.6

Sulphur Dioxide Exposure

Subjects	Pre-Expos.	Post-Expos.	% Change	15' Post.
1. J.C.	0.4	0.9	150	0.5
2. R.D.	0.6	1.1	83	0.6
3. R.M.	0.3	0.8	166	0.4
4. P.B.	0.5	1.2	140	0.5
5. J.B.	0.6	1.2	100	0.5
6. B.B.	0.5	0.9	80	0.4
7. R.A.	0.7	1.3	86	0.8
8. J.D.	0.5	1.5	200	0.7
			Mean 126%	
			S.D. 45%	

unchanged from pre-exposure levels.

Two of the six subjects (J.D., P.B.) reported 50ppm. sulphur dioxide to be slightly irritant. No subjects reported the subjective sensation of tearing or ocular anesthesia.

The Effect of Prostaglandin Synthesis Inhibition on the Irritant Response

The results of this study are presented in Table 5.4.

Four of the six subjects in this study reported gastrointestinal side-effects from oral indomethacin. The most common side-effects noted were an "upset stomach", "heartburn", and a "cramp"-like upper abdominal distress. These symptoms were most severe with the first dose of indomethacin, decreasing somewhat with the next three doses. Subjects were directly observed taking the first and last doses; in spite of the side-effects, it is believed that the subjects' reports of taking the second and third doses are credible.

Exposure to 50ppm. sulphur dioxide two hours after the final indomethacin dose produced a characteristic tearing response. The mean percentage increase in tearing immediately post-exposure was 123% ± 50%. Following exposure the tearing response resolved rapidly and in all cases, by fifteen minutes post-

Table 5.4

The Effect of Indomethacin Pretreatment on the Tearing Irritant Response (mg. tears/15 sec.)

Subjects	Pre-Expos.	Post-Expos.	% Change	15' Post.
1. J.C.	0.6	1.3	117	0.7
2. R.M.	0.5	0.9	80	0.4
3. P.B.	0.6	1.1	83	0.6
4. B.B.	0.3	0.8	167	0.3
5. W.C.	0.9	1.7	89	0.7
6. M.W.	0.4	1.2	200	0.5
		Mean	123%	
		S.D.	50%	

exposure, tearing had returned to pre-exposure levels.

Three of the subjects in this study (P.B., W.C., M.W.) reported the subjective sensation of ocular irritation with sulphur dioxide exposure; no subjects reported the sensation of tearing.

The Effect of Trigeminal Spinal Tractotomy on the Irritant Response

All three of the subjects in this study appeared to have increased tearing in the affected eye following exposure to freshly cut onion vapour. Although this is an interesting, potentially meaningful result, its statistical significance is unclear because of the sample size.

The Effect of Chemical Ocular Irritation on Heart Rate

The results of this study are presented in Table 5.5.

No meaningful change was found in heart rate following ocular exposure to 50ppm. sulphur dioxide.

One of the subjects in this study (V.H.) reported the subjective sensation of eye irritation with sulphur dioxide exposure; no subjects reported the sensation of tearing.

Table 5.5

The Effect of Chemical Ocular Irritation on Heart Rate

	Heart Rate			
Subjects	Pre-Expos.	1' Expos.	5' Expos.	5' Post. Expos
1. J.C.	66	69	69	70
2. R.D.	82	80	79	76
3. B.G.	83	83	81	79
4. V.H.	90	81	80	81
5. R.S.	62	63	74	66
6. S.A.	75	78	79	72
7. P.A.	74	66	82	71
8. R.E.	83	78	71	72
9. C.H.	71	75	73	74
10. M.T.	79	80	84	77
Mean	76.5	75.3	77.2	73.8

Discussion

The Effect of Contact Lenses on the Irritant Response

In this study it was found that impermeable contact lenses completely blocked the tearing response to 50ppm. sulphur dioxide. Because the lenses only shield the central portion of the cornea, this finding strongly suggests this location for the ocular "chemical sense" receptors rather than the conjunctiva or corneal margins. The central corneal location for the chemoreceptors is also supported by neuroanatomical and physiological studies showing this area to be the most sensitive portion of the eye (Norn 1973), and the site with the highest density of nerve endings (Matsuda 1968, Fozsa and Beuerman 1982).

To reach the corneal receptors, irritants must first dissolve in (or be suspended in) the tear film; they then would either directly contact the corneal epithelium or be carried to the cornea by tear flow and blinking. Thus, the tear film plays a primary protective role by both buffering and diluting noxious environmental agents. The addition of a well-fitting contact lens further protects the cornea by both preventing irritants from passing directly through the thin pre-corneal tear film, and by limiting free access

of irritant-laden tears to the cornea.

From studies cited earlier showing only limited tear flow under the contact lens with each blink, it seems reasonable to assume that the lens' shielding ability would not be absolute. For example, at high irritant concentrations--concentrations high enough to overload the buffering and dilutive capacity of the tear film--a quantity of irritant sufficient to stimulate the irritant response might still reach the chemosensitive areas of the cornea in spite of the lens. In effect, then, contact lenses alter the irritant response by increasing the response threshold rather than blocking the response entirely.

The highest sulphur dioxide concentration allowed for use in the present study was only 50ppm., a level at which contact lens wearers did not report the subjective sensation of eye irritation or show a measurable irritant response. How high a concentration would be necessary to overload the contact lens shielding effect is unknown and, to date, has not been addressed in the ophthalmological literature. However, anecdotal reports suggest that impermeable contact lenses actually offer relatively little protection for potent lacrimators (CBMN, for example), and moderate exposures to these agents can stimulate gross tearing (Ward 1984). This observation points to the importance

of the irritant's specificity: for agents that primarily stimulate the tearing response (like the lacrimators, and opposed, for example, to general sensory irritants such as sulphur dioxide), the contact lens shielding effect may be easily overcome because even the small amounts reaching the cornea are sufficient to stimulate tearing.

As mentioned in Chapter 4, the tearing response to both sulphur dioxide and ammonia was found to be concentration- rather than dose-dependent. In future investigations it would be useful to study the tearing responses to dose-dependent irritants (such as formaldehyde or acrolein) with contact lenses in place. With these agents, Douglas (1975) found that a characteristic product of irritant concentration and exposure time (C x T) was necessary before the sensation of irritation was perceived. The molecular mechanism of action of the dose-dependent irritants is unkown, but it seems likely that they must be unbuffered (or only partially buffered) by tears and either accumulate in the tear film or adsorb onto the corneal surface until a sufficient level is reached to precipitate the irritant response. If this is the case, using these agents it should be possible to provoke an irritant response in contact lens wearing subjects simply by sufficiently lengthening the exposure period. Comparison of an

individual subject's C x T product with and without contact lenses would then give a direct indication of the contact lens shielding effect.

The current study's contact lens results are also interesting because they again support the existence of an ocular "common chemical sense". A number of studies cited earlier found a decrease in pain-touch sensitivity in contact lens-wearers' eyes. However, in the present study these erstwhile "insensitive" eyes showed a sensitivity to chemical irritants (as indicated by the percentage change in the tearing response to 50ppm. sulphur dioxide) approximately equal to that of non-contact lens wearers. This finding would seem to suggest that the chemical irritant response can be separated from the eye's responses to pain and touch. And, if this is the case, the ocular irritant response may, indeed, be another manifestation of a separate common chemical sense.

At least two possible neural mechanisms might explain the separation of pain and touch from ocular chemical perception. First, as suggested by Keehle (1964), Montcrieff (1967) and others, there could be several specific receptor populations among the mass of corneal unmyelinated nerve endings. Although these nerve endings appear to be similar microscopically (Lele and Weddell 1959, Matsuda 1968, Tanaka 1973,

Tervo et al 1979, Schimmelpfennig 1982), functional differences may exist, with some being specialized chemoreceptors while others are specific for pain or touch. Another possibility is that the corneal free nerve endings are, in fact, as undifferentiated in function as they appear to be in structure, and that different types of stimuli are only "recognized" in the central nervous system by some pattern in the afferent imput.

To date, experimental evidence has not been helpful in resolving this fundamental problem. Recordings made from single corneal sensory fibers have shown different electrical responses for mechanical and thermal corneal stimulation (Lele and Weddell 1959, Tanaka 1973), but the intrinsic variation of the response was large and no studies have yet specifically focused on corneal neural responses to irritant gases. Further progress here will have to await more neurophysiological investigations of the effects of chemical irritants and ever more sensitive neurological recording techniques.

Finally, it is both interesting and reassuring that the tearing levels measured in the present study's contact lens-wearing subjects were similar to those found by Farris et al (1981). In the earlier study, no differences were found in the basal tear volumes and

reflex tear flow rates of contact lens-wearers and normal controls. This corresponds well with the present study's finding of similar ranges of pre-exposure tearing levels and irritant responses in both contact lens-wearing and non-contact lens-wearing subjects.

The Effect of Beta-Adrenergic Blockade on the Irritant Response

Pretreatment with one drop of 0.25% timolol solution produced no measurable effect on the tearing response. This dose of timolol was known to effectively block beta-adrenergic receptors in the anterior chamber and conjunctiva (Neufeld et al 1983). Thus, its failure to alter the irritant response suggests that ocular beta-adrenergic receptors are not involved in this process.

In interpreting this result, however, several additional considerations should be kept in mind. First, the true function of the human ocular beta-adrenergic receptors remains unknown (Bron 1969, Neufeld et al 1978). In the absence of this knowledge, it is impossible to be certain that beta-blockade does not interfere with any aspect of the irritant response. Secondly, to give consistency to this series of afferent pathway studies, the same irritant (50ppm. sulphur dioxide) was used in each. Again, not knowing the

function of the ocular beta-adrenergic system, it is possible that the concentration may have been either too high or low--that, perhaps, blockade of these receptors would have a recognizable effect only at relatively high or low sulphur dioxide concentrations, but not at the 50ppm. concentration chosen. Finally, in a similar vein, it may be that beta-adrenergic receptors only participate in the irritant response for one or a small group of substances (for example, the lacrimators).

These reservations stated, there does exist some theoretical and experimental support for the argument that the beta-adrenergic ocular receptors blocked by timolol are not involved in the irritant response. For example, in isolated human and rabbit corneal preparations, sodium and chloride transport has been shown to be affected by the balance between alpha- and beta-adrenergic influences (Wiederholt et al 1983). This quite unphysiological investigation tells little about beta-adrenergic function under <u>in vivo</u> human conditions, but it at least suggests a useful alternative role for this ocular receptor system.

Of greater importance may be that, in both normal controls and glaucoma patients, chronic timolol use does not seem to produce any abnormalities in human tear production as measured by the Schirmer test

(Kitazawa and Tsuchusaka 1981). As discussed earlier, the Schirmer test procedure mechanically irritates the eye. Although mechanical irritation may involve an entirely different perception and response process than chemical irritation, the tearing responses found with mechanical and chemical ocular irritation are similar (Mishima et al 1966). Thus, Kitazawa and Tsuchusaka's study of the effect of timolol on the Schirmer test also would seem to support the current study's finding that ocular beta-adrenergic receptors are not involved in the irritant tearing response.

The finding that most subjects in the present study did not consider timolol to be an irritant or anesthetic agrees with the earlier subjective findings of Katz et al (1976). The fact that tearing levels measured five minutes after timolol instillation were unchanged from pre-timolol levels, provides further objective confirmation for these subjective reports. Draeger et al's 1983 study of the local anesthetic action of timolol showed it to be associated with a transient (two to three minute) decrease in corneal sensitivity. From this report (and the present study's finding of a similarly brief period of irritation from the local anesthetic benoxinate) it seems reasonable to assume that if timolol actually had a direct irritant effect, it would also be expected to resolve within a

few minutes. Thus, the present study's finding of no tearing increase at five minutes post-timolol is not surprising, although slight increases may have been found if tearing had been measured at one or two minutes after timolol exposure.

The Effect of Prostaglandin Synthesis Inhibition on the Ocular Irritant Response

Indomethacin inhibition of ocular prostaglandin synthesis had no measurable effect on the ocular irritant response. This finding is of particular interest because it allows the irritant response to be related to the ocular inflammatory response. As discussed in Chapter 2, ocular inflammation from topical nitrogen mustard, high concentrations of formaldehyde, paracentesis, or laser burns, has been shown to occur in two phases: an immediate, neurally-mediated flare of increased intraocular pressure quickly resolves and is followed, in one to two hours, by a prostaglandin-mediated, prolonged anterior chamber reaction with increased intraocular pressure, miosis, and loss of the blood-aqueous barrier. It is only the second phase that can be blocked by prostaglandin synthetase inhibitors such as indomethacin.

This distinct temporal and chemical difference in pattern of response is useful in characterizing the tearing irritant response. In contrast to the complex

pattern of inflammatory changes, the present study has found the ocular irritant response to be a simple, rapid reaction that, when provoked in only one eye, also occurs almost immediately in the contralateral eye. The failure of indomethacin pretreatment to alter the irritant response strongly argues against the involvement of prostaglandins. Taken together, these three characteristics of the irritant response--the speed of onset of the response, the contralateral effect, and the lack of prostaglandin involvement--suggest a neurological mechanism. And, if this is the case, the ocular irritant response would appear to be similar to (in fact, indistinguishable from) the initial, neural phase of the ocular inflammatory response.

Postulating a neural mechanism for the ocular irritant response also provides a reasonable biochemical explanation for one of the fundamental characteristics of irritant chemicals--their ability to produce different effects at different concentrations. As mentioned in Chapter 2, Henderson and Haggard (1943) defined irritants as substances that cause reversible aversive responses at low concentrations while producing pathological changes at high concentrations. This characteristic is well demonstrated by sulphur dioxide and ammonia, both of which can cause permanent ocular damage at high concentrations (Grant 1974,

Wright 1982), and can also be seen with dose-dependent irritants like formaldehyde (Butler et al 1979), and specific lacrimators (Pfannkuch and Bleckman 1982).

The neural phase of inflammation has been shown to be associated with the release of neurotransmitters like Substance P into the aqueous. These active peptides presumably originate from the action of the toxic agents on the cornea and other anterior chamber structures, and aqueous SP levels have been found to be directly related to the concentration of the agent causing the inflammation (Nishiyama et al 1981). In view of this, it is likely that there exists an SP "threshold" for the induction of the inflammatory response: low concentrations of irritants would cause the release of relatively small, sub-threshold levels of SP, producing only the short-lived, reversible, irritant-like response; higher irritant concentrations (in the range known to cause pathological change) would produce large amounts of SP and, as aqueous SP levels rose above the critical response threshold, the full-fledged inflammatory response would be precipitated.

Although attractive, this explanation remains speculative and is likely to be an oversimplification. For example, as mentioned previously, SP has been shown to be only one of a number of mediators of ocular inflammation, and it may be that inflammation is act-

ually precipitated by a complex "cascade" of active substances. However, such a model does provide a biochemical link between ocular irritation and inflammation--two phenomena that have long recognized as being clinically related--and also suggests a direction for further experimentation.

The SP-threshold mechanism of irritation and inflammation could be evaluated in several ways but, at present, none seems suited for use with human subjects. First, the effect of SP-antagonists and nerve blocking agents on the irritant response could be studied. In the past decade a number of SP-antagonists have been synthesized, most being substituted SP molecules such as (D-Pro^2-D-Trp^{7,9})-SP or (D-Pro^2-D-Phe^7, D-Trp^9)-SP (Folkers et al 1981). These agents have had variable effects in animals, depending on the species tested, specific ocular response studied, and method of tissue treatment (Butler et al 1981, Holmdahl et al 1981, Mandahl and Bill 1983). But, these methodological problems aside, a study utilizing SP-antagonists might help to clarify the nature of the ocular response's neurochemical "trigger".

Perhaps even more fundamental might be a study of nerve blocking agents on the tearing response. Here, specific neurotoxins (tetrodotoxin or saxitoxin) and high doses of topical anesthetics could be used to

confirm that the tearing response is truly neurally-mediated. In the cat, for example, it has been shown that tetrodotoxin and high doses of benoxinate can block the early neural phase of inflammation and prevent the development of the inflammatory response (Unger et al 1977, Duffin et al 1982). The use of these techniques to study the irritant response would be helpful because the toxins' mechanisms of action are well understood (Kao 1965, 1967, Narahashi 1972) and, because of this, any findings would be particularly meaningful.

A second potentially useful approach might be to expose the eyes of animals to increasing concentrations of an irritant while anterior chamber intraocular pressure, SP, and PGE_2 levels were monitored. The results of this study should allow direct definition of the "inflammatory threshold", and whether the putative threshold is SP concentration- or dose-dependent. The design of such an exposure study might be complex: for example, to avoid confounding from adaptation to the irritant and the trauma of repeated aqueous sampling, each irritant concentration might require a different animal (or, more properly, group of animals) making base-line definition difficult. But, this type of study would be particularly important because it would directly analyze the intermediate steps in the ocular

irritant response.

The Effect of Topical Anesthesia on the Ocular Irritant Response

As mentioned above, high doses of topical anesthetics like benoxinate are capable of inhibiting the ocular inflammatory response. However, in the present study a small (though definitely anesthetic) dose of this agent had no measurable effect on the irritant response. Because topical anesthesia is dose-related (Polse et al 1978, Draeger et al 1984), a difference in response with different doses is expected. But the results of the current study are interesting because they differ from those of earlier high-dose anesthetic studies, and also provide further insight into the mechanism of ocular chemoreception.

In the very different studies of Mutch (1944), Butler et al (1979), and Duffin et al (1982), pretreatment with topical anesthetics was found to inhibit the ocular response to "phenyl-bromo-aceto-nitrite" (a "tear gas" supplied to Mutch by the War Office), neutral formaldehyde solution applied directly to the eye (Butler et al), and mechanical trauma to the iris (Duffin et al). The doses of anesthetic used in these studies were relatively large: "several drops of 4% cocaine" (Mutch), one drop of 0.4% benoxinate every two and a half minutes for thirty minutes (Butler et al),

and one drop of 0.4% benoxinate every five minutes for thirty minutes (Duffin et al).

In contrast, in the present study only one drop of 0.4% benoxinate was used in each eye. This small dose of benoxinate was, however, definitely sufficient to produce ocular anesthesia--an observation confirmed by Polse et al's 1978 ocular anesthetic dose-response studies. The anesthetic doses used by Mutch, Butler et al, and Duffin et al were much larger than those required for anesthesia alone, and undoubtedly induced a pharmacological nerve block similar in effect to that caused by nerve toxins.

That a "lightly" anesthetized eye still responded to 50ppm. sulphur dioxide would again seem to suggest the presence of an ocular chemical sense separate from pain and touch. But, given that a single drop of benoxinate would contact all the superficial sensory neurons in the cornea, some basis must be proposed for the selective effect of topical anesthetics on pain and touch but not on chemical sensation.

Several mechanisms for this type of differential block have been suggested by Franz and Perry (1974). First, among the corneal sensory neurons there might be slight but significant differences in axon diameter, with smaller axons being blocked more rapidly than larger fibers. Secondly, there may be differences in

exposed axon length, with smaller exposed areas being blocked more completely. For example, it could be that the neurons associated with chemosensation are either somewhat larger, or lie in slightly deeper or more shielded locations in the complex surface of the corneal epithelium (Schimmelpfennig 1982, Nichols et al 1983), than the neurons for pain and touch. Alternately, if afferent information for several "senses" comes from the same receptor (with chemical "sensation" a function of central nervous system signal processing), it could be that differences in extent of blockade alter the pattern of impulses presented to the central nervous system. Pattern changes of this type may be sufficient to interfere with pain perception but still retain enough "information" for chemoperception.

Finally, the present study again confirms that benoxinate is an ocular irritant. Initial descriptions of this agent (Schlegel and Swan 1954) characterized it as less irritant than other available anesthetics, but later studies called this into question (Linn and Vey 1955). The benoxinate tearing response found in the present study showed its irritant effect to be mild and brief. After benoxinate instillation, tearing always rapidly returned to pre-exposure levels and, in all cases, the tearing level stabilized before sulphur dioxide exposure.

The Effect of Trigeminal Spinal Tractotomy on the Ocular Irritant Response

In subjects who had undergone trigeminal spinal tract section, the tearing irritant response appeared to be essentially intact. As noted previously, the number of subjects in this study was small, so this finding cannot be considered significant. In addition, both the irritant employed and tearing assessment method were different from those used in the other phases of this study, making direct comparison impossible. Nevertheless, the findings from the three subjects will be considered briefly because they support further speculation about the central nervous system mechanisms involved in the ocular irritant response.

Very little has been published about the ocular irritant responses of individuals who have surgical, traumatic, or congenital trigeminal abnormalities. Two studies, however, are of relevance--although neither directly addresses the question of the site of central integration of ocular chemosensory information.

First, in 1944, Mutch studied the tearing responses of an individual who had undergone section of the "sensory root of the fifth nerve" on the left side as a treatment for trigeminal neuralgia (the details of the surgical procedure are not given but, presumably,

the section was performed just distal to the trigeminal ganglion). When Mutch protected the right eye and directed a "tear gas" (again, "phenyl-bromo-aceto-nitrite") into the left eye, there was no tearing response; when the right eye was unshielded and tear gas directed into both eyes, only the right eye was painful and teared. (Unfortunately, Mutch never carried out the third possible exposure--of the left eye alone, watching for tearing in the right eye--which would have revealed whether the left eye had an efferent and/or afferent block, and also provided more information about contralateral responses.) In this case, Mutch reasonably concluded that the left eye's tearing response was lost due to complete interruption of the afferent sensory pathway. In terms of the present study, this patient's condition would be analogous to the sulphur dioxide-induced tearing response either after pretreatment with nerve blocking agents (tetrodotoxin or high doses of topical anesthetics) or with contact lenses shielding the cornea. And, it was specifically to prevent this type of functional loss (often with associated keratitis) that the more proximal surgical procedures, like trigeminal spinal tractotomy, were devised. In Mutch's case it is also possible that, as a consequence of the surgical procedure or some later trophic change, the left eye's efferent

pathway was also affected, further obscuring the study's interpretation.

Secondly, several studies have been made of tearing in animals following intracranial trigeminal nerve section (Alper 1975) or radio-frequency thermocoagulation of the trigeminal ganglion (Schimmelpfennig and Beuerman 1982). In these cases, no differences were found in the "basal" tear production of the denervated and control sides. However, it should be kept in mind that tearing levels were not objectively measured in these studies and, importantly, physical or chemical ocular irritation tests were not undertaken. Thus, the effect of these trigeminal procedures on the tearing response to chemical irritants remains unknown.

In the present study, the tear film of the anesthetic eye also appeared normal prior to stimulation. In response to onion vapour irritation, the lower meniscus of the anesthetic eye was observed to increase slightly in size--an early change associated with increased tear production (Holly and Lemp 1977). This finding suggests that the trigeminal nerve's spinal tract is not necessary for the ocular chemical irritant response and, consequently, that this sensation is probably a function of the main sensory trigeminal nucleus.

However, in interpreting this study there are a

number of concerns beyond the small sample size and subjective nature of the observation. First, the participants in the present study may not be a representative sample of all tractotomy cases. For example, many published studies have noted considerable variability in the trigeminal pathways, with fibers carrying sensory information for touch (a function traditionally associated with the main sensory nucleus) crossing over into the spinal tract. Because of this, it is possible that some chemosensory information may be carried in either tract, and that the irritant response may not be an exclusive function of the main sensory nucleus. Those in whom it is carried in the spinal tract (and lost with tractotomy) may, in some way, be more prone to develop complications necessitating other surgical procedures, and consequently either have a higher mortality or no longer be classified as "tractotomy patients" in hospital records. In contrast, the patients in the present study all had a long-term, successful outcome, and the role of the main sensory tract in their ocular chemosensation may be the exception rather than the rule. If this is the case, then "crossed fibers" in the trigeminal tract could form the basis for a sampling error.

Secondly, the exact location and extent of the subjects' trigeminal tract lesions are unknown, and

could only be fully determined at autopsy. This problem was as a major concern of the surgeons who pioneered the procedure, and its resolution only seems possible with an investigation using experimental animals. Building on the work of Schimmelpfennig and Beuerman (1980, 1982), for example, stereotactically-placed lesions could be made, the ocular irritant response tested, and the lesion site and extent confirmed at autopsy. Although such a study would involve intra-species generalization, it is hoped that it will be undertaken by neurosurgeons or ophthalmologists because only this type of experimental approach could definitely determine the intracranial pathway for the chemical irritant response.

There currently exist some clinical techniques, however, that might be useful in studying the afferent response pathway in human subjects. The use of advanced E.E.G. recording techniques (BEAM--brain electrical activity mapping--machines) during chemical eye irritation might yield characteristic response patterns that could be related to one or the other trigeminal nuclei. Further definition might also be possible by use of response-summation techniques, such as the recording of sensory-evoked potentials. These procedures would be relatively acceptable to volunteers, eliminating the difficult task of finding and recruit-

ing post-tractotomy patients (with all the attendant uncertainty about lesion anatomy) and using data from other species.

The Effect of Ocular Irritation on Heart Rate

In the present study no relationship was found between heart rate and ocular irritation with 50ppm. sulphur dioxide. This result is most likely due to an inadequate level of ocular stimulation. As was noted previously, no change in heart rate was found in Ben David's 1977 study in which the eyes were exposed to 168ppm. sulphur dioxide. And, in both Ben David's and the present study, the irritant concentration was not sufficient to consistently cause subjective symptoms of eye irritation. However, higher levels of mechanical stimulation and, in Ben David's work, even air flow at approximately one liter per minute, did cause measurable bradycardia. To better define the relationship between ocular irritation and heart rate and to confirm the already known trigeminal-vagal relationship, studies monitoring heart rate during brief exposures to higher concentrations or higher total doses of irritants should be undertaken in volunteers and experimental animals.

CHAPTER 6

CONCLUSION: OCCUPATIONAL IMPLICATIONS OF THE OCULAR IRRITANT RESPONSE

One of the major concerns of occupational health workers is the setting of rational industrial hygiene standards. Unfortunately, this process of defining acceptable exposure levels for environmental chemicals has not been an easy task, and the standard-setting process has often been justifiably criticized. Authors previously cited, for example, have described the current "consensus" method of arriving at threshold limit values as "arcane" (Douglas 1975), "trial and error" (Hatch 1972), "easily subject to misinterpretation" (Stokinger 1969), and "more of an educated guess or an art than a science" (Holmberg and Winell 1977). Among the most fundamental deficiencies in the standard-setting process are the lack of objective human exposure data and the lack of an adequate understanding of the effects of environmental chemicals on human physiology. This type of information is necessary not only for definition of an acceptable exposure range, but

also (and perhaps even more fundamentally) for selection of an appropriate biological response (tearing, bronchoconstriction, respiratory rate) upon which the standard is to be based.

In this concluding chapter, the implications of the present study for these more general occupational concerns will be considered, beginning with the problem of defining the ocular "threshold" for irritant chemicals.

The Ocular Tearing Threshold

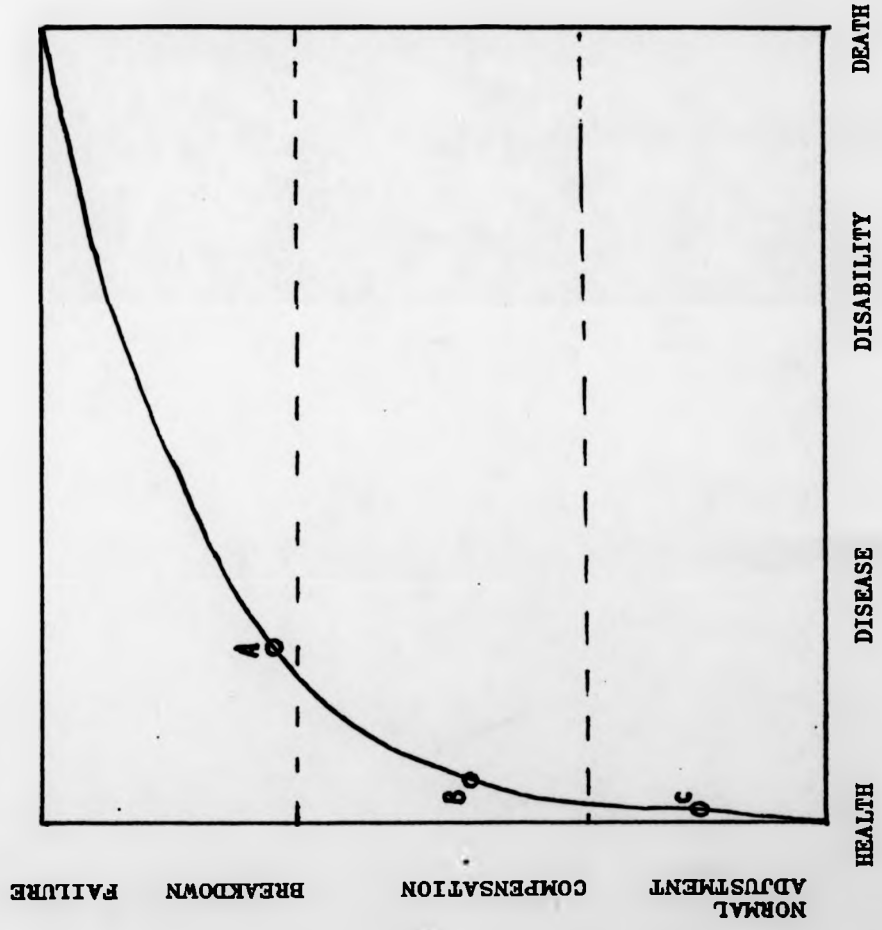
The theoretical basis for the determination of threshold limit values has been repeatedly reviewed (Stokinger 1969, Hatch 1972, Holmberg and Winell 1977, Thomas 1979, Henschler 1984, Paull 1984). To summarize briefly, for each chemical there is believed to be a measurable exposure level--a "threshold"--below which individuals are either unaffected or are able to compensate for any ill-effect without "impairment of health", but above which there occurs short- and/or long-term "injury". Of all the vague concepts in this statement, perhaps the most fundamental is that of the "threshold" itself--the imaginary point on the continuum of "health" defining the acceptable exposure level.

For a threshold limit value to be meaningful, it must be based on measurement of an appropriate acute response. Criteria for response selection have been reviewed by Hatch (1972): as might be expected, most important are, first, that the response has a predictive value--that it is in some way related to (possibly, an indicator of) of impending ill-health and, second, that a quantitative relationship exists between exposure and response (ideally, with the response terminating before the dose falls to zero).

Hatch presented these concepts in chart form (Fig. 6.1). In this chart, the human physiological response to environmental chemicals ranges from "normal homeostatic adjustment" to "compensation", "breakdown", and finally "failure" (the development of pathological change in the organism). Behaviorally, the physiological changes are paralleled by a change from "health" to "disease", "disability", and finally death. According to this model, the critical threshold lies between the stages of "compensation" and "breakdown", at the border between "health" and "disease"; the maximum exposure limit would ordinarily be set at the "limit of the compensatory zone" (point A) or, to give an added measure of safety and allow for human variability, slightly lower in the compensation zone (point B, for example).

Fig 6.1 Impairment - Disability Relationship Curve

(from Hatch, 1972)



Unfortunately, in real life the determination of this threshold becomes quite complicated. For example, one key problem is defining of the point at which "ill health" begins. On this question there are major conceptual differences between the West (Western Europe and the United States) and East (Eastern Europe and the U.S.S.R.). In the West, the threshold is usually defined as the level at which morphological changes associated with a recognized disease first appear (Zielhuis 1974). According to this concept, chemical exposures producing characteristic, organ-specific effects (for example, tearing with ocular irritant exposure) would be acceptable as long as they were either within the range of "normal" physiological variation, or were adequately compensated so that tissue damage did not ensue.

In contrast, although the current practice is reported to be changing, Eastern exposure limits are commonly based on the level at which the first functional response is detected (point C, Fig. 6.1). According to this philosophy (characterized by Hatch as the "qualitative" approach to toxic reactions), this earliest response is viewed as a definite warning of impending injury and marks the beginning of "ill health".

These different approaches to hygiene standards have had a variable effect on threshold limit values in the East and West. For some substances (approximately twenty compounds) the levels are similar; but for a larger number (approximately fifty compounds) the acceptable exposure levels are considerably lower in the East--occasionally by a factor of fifty-fold or more (Holmberg and Winell 1977).

The present study's objective ocular irritant response data bears directly on this ongoing international debate. As has been described, the tearing irritant response appears to be a neurogenic reflex. That the response is within physiological limits is confirmed by the significantly larger changes in tearing caused by non-noxious environmental stimuli. For example, as noted previously, wind, mild physical stimulation (rubbing, or consciously holding the eyes open), and emotional tearing can produce much greater tearing increases than the 100-300% found with sulphur dioxide and ammonia (Mishima et al 1966, Holly and Lemp 1977). Thus, the tearing response to ten or more times the currently accepted threshold limit concentrations for common irritants is still well within the range of normal physiological change. In view of this, the tearing response thresholds found in the present study would either lie within the "zone of normal adjustment"

or, at worst, in the "zone of well-compensated change". (In contrast, the threshold for the ocular inflammatory response would be found in the "breakdown" area, just beyond the limit of the "compensation zone").

The "normalicy" of these in vivo human ocular findings would seem to be a strong argument against the Eastern practice of defining the exposure limit as the level at which any change is first detectable. However, in interpreting this data several questions must be considered. Most important is whether the tearing response is an "appropriate" indicator--is it actually associated in some way with "impending ill health"?

At first glance, the physiological range of irritant-induced tearing would seem to make this and unlikely conclusion. However, as was discussed in Chapter 3, one of the major functions of tearing is to protect the eye. Aside from its role in buffering and diluting (literally "washing away") noxious environmental substances, the protective function of tearing is also implied by its quantitative relationship to irritant concentration. Furthermore, as can be seen in the response curves, the tearing response ceases well before the irritant dose falls to zero, with the derived response thresholds comparing quite favorably with those of other critical organs such as the lung. Thus, in its protective function--sensitively tuned to

the level of environmental "threat"--and its link to the well-accepted pulmonary irritant response, the ocular tearing system would be reasonable to use as a basis for threshold limit value definition.

A second reservation stems from the present study's use of relatively brief exposures. These exposures are analogous to acute irritant exposures in the workplace but cannot provide insight into the effect of adaptation, or the likely outcome of chronic ocular exposure. To address these questions would require a different experimental design or a large-scale survey of chronically-exposed workers.

A clue as to the possible outcome of chronic ocular irritant exposures can be found in studies of chronic pulmonary exposures. In the lung, it has been found that long-term exposure to low levels of sulphur dioxide can be associated with chronic lung changes (Smith et al 1977). The threshold for this chronic effect is 1ppm.--the same concentration that Douglas (1975) and others found to be the threshold for acute bronchoconstriction. Anatomical studies discussed earlier suggested that the pulmonary response to acute sulphur dioxide exposure is similar to that of the eye, and that the ocular and respiratory tract chemoreceptors are similar in structure and function. By analogy, then, it might also be expected that the

sulphur dioxide threshold for chronic ocular change would be approximately the same as that found for the acute tearing response, 5 ppm.

To date, no specific chronic ocular irritant exposure studies have been undertaken that could confirm the acute-chronic threshold relationship. This information, however, would be quite valuable. For example, if chronic sulphur dioxide exposure at concentrations at or above the 5 ppm. acute response threshold are found to be associated with chronic eye disease (as may be the case with the lung), it would imply that the acute threshold is actually in the "breakdown" or "decompensation" zone rather than the zone of "normal" or "compensated" response. And, if this is the case, it would further suggest either that the acute and chronic thresholds need to be defined separately, or that the Eastern concept of "threshold" may be correct--that the earliest change from the normal, unstimulated state may indeed mark the onset of long-term pathology or "ill health".

Definition of the chronic ocular irritant threshold would require a study of the relationship between chronic ocular change and environmental irritant exposure. In animals such a link has been suggested by histological abnormalities in the conjunctival epithelium of hampsters chronically exposed to cigarette

smoke (Basur and Basu 1980). Although no similar human data is currently available, this question might be approached by studying the relationship between cigarette smoke (a known irritant containing, among other substances, formaldehyde and acrolein--see Ayer and Yeager 1982) and chronic eye disease. Such an investigation has been planned involving both an experimental study of the ocular irritant effects of cigarette smoke and a case-control study of cigarette smoking and eye disease (the prospectus is included in the Appendix). It is hoped that the study will be undertaken in the near future and will provide new insights into the meaning and long-term significance of the ocular tearing threshold.

The Use of Contact Lenses in Industry

The ocular irritant response and tearing measurement method described here can also be used to study specific problems of occupational interest. This potential is well illustrated in the question of whether contact lenses can be safely worn at work.

In most industries, employees are not permitted to wear contact lenses (Council on Occupational Health 1964, Fox 1967). Although it is widely acknowledged that visual acuity with contact lenses is often superior to that with spectacles (peripheral vision, in

particular, being much improved), there is an unstated, but common fear that foreign bodies or chemicals will accumulate, or lodge behind the lenses, causing greater damage than might be expected without contact lenses.

Dubbed the "chemical contact lens problem" (Guthrie and Seitz 1975), this rationale for the restriction of contact lens wear has been repeatedly questioned. For example, animal studies have shown that impermeable contact lenses actually protect the eye against splashes of irritant liquids or showers of burning grit particles (Guthrie and Seitz 1975, Nilsson et al 1981, Nilsson and Andersson 1982). However, no in vivo human data on this question was available until the present study's finding that contact lenses blocked the tearing response to irritant gases. Based on this confirmation of the "protective" effect of contact lenses, it can now be more confidently suggested that, given the use of suitable eye protection in areas of risk, there is no fundamental reason to prohibit the use of contact lenses at work.

Again, this finding is a demonstration of the use of the techniques of the current study to answer an occupationally important question; the availability of such in vivo human data gives the results an immediate relevance for occupational health and environmental regulation.

The Collection of In Vivo Human Data

The present study has shown that it is possible to safely and acceptably obtain *in vivo* human exposure data using relatively simple techniques. And, as discussed in Chapter 3, the measuring method employed can undoubtedly be improved. However, the use of human beings in exposure studies has obvious ethical and practical limitations. Clearly, only acutely toxic agents whose acceptable exposure range is already known can be studied. For unknown substances, or those that may have a chronic effect, human studies can only be used to confirm that the exposure range found in animal studies is appropriate for human beings. Finally, volunteer human exposure studies also have the practical drawbacks of being difficult to organize and relatively time consuming to perform.

In spite of the distinct regulatory need for human exposure data, then, theoretical and practical considerations limit its collection. In the absence of major technological innovations in exposure or measuring methodology, it is probably unrealistic to expect great increases in the availability of human exposure data, leading to the question of the role human testing will ultimately play in the standard-setting process.

The scope of the problem facing those responsible for environmental chemical regulation is enormous.

For example, a recent report by the National Research Council (1984), noted that there was sufficient health hazard data for only 30% of more than 50,000 common chemicals, pesticides, drugs, and cosmetics sold in the United States. No data at all was available for 40% of the pesticides and 25% of drug ingredients. Of equal concern was that, for almost 80% of all substances neither ingested nor worn, there was absolutely no toxicological data.

Given this situation, it is unlikely that the haphazard, consensus method of standard-setting will ever be adequate. Two general directions of change are forseeable and, in each, human exposure testing such as that used in the present study will play an important role.

First, efforts are currently underway to develop objectively-measurable chemical testing techniques using simple biological systems (bacteria, cell cultures, isolated muscle strip preparations). These test systems have the advantages of simplicity, speed, relatively low cost per test, and freedom from ethical restraints. But, because they are far removed from the complex interrelationships found in the human organism, the results of these test systems will continue to need the type of selective confirmation only available from direct human exposure testing.

The second direction for development is more complex but may, ultimately, hold even greater promise. From both human and animal exposure studies, it may finally be possible to develop a series of truly meaningful structure-activity relationships. This knowledge of the principles underlying chemical activity would allow prediction of toxicity on the basis of chemical and physical structure alone--a procedure ideal for computerization. Agents passing such an initial screening would still require exposure testing in simple biological systems, experimental animals and, for some, human experimental exposures. But, if the basic structure-activity relationships were thoroughly understood, the need for human testing would be less pressing, and it could be reserved for "quality control" in the standard-setting system.

Preliminary work on the development of structure-activity relationships has proceeded haltingly for the past century (see reviews in Alarie 1973, Douglas 1975, 1980, Steinhagen and Barrow 1984). Studies are now being planned using the ocular tearing response and measuring method described in this study to continue the investigation of this relatively little known but important aspect of occupational health. In this way it is hoped that the limited availability of human experimental ocular exposure data can best be employed

for the benefit of environmental standard development and, ultimately, the prevention of occupational disease.

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APPENDIX

THE IRRITANT EFFECTS OF CIGARETTE SMOKE ON THE HUMAN EYE: A PHYSIOLOGICAL AND EPIDEMIOLOGICAL PILOT STUDY

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Summary

The purpose of this study is to investigate the acute and chronic irritant effects of cigarette smoke on the human eye, and to determine whether cigarette smoking may be associated with chronic external eye disease. To examine the relationship between cigarette smoking and eye irritation, tear production will be measured before and after exposure of the eyes of smoking and non-smoking volunteer subjects to main- and side-stream cigarette smoke and individual smoke constituents. The possibility of a link between cigarette smoking and chronic eye disease will be investigated by means of a case-control study. In this study, the smoking habits of patients with chronic external eye diseases will be compared to those of individuals with squint and accomodation problems, as well as with patients having diabetic retinopathy--a condition that has been shown to be unrelated to cigarette smoking. Controls will be matched to cases by age and sex. Inquiry will be made into the attitudes of all subjects towards cigarette smoking, and it is hoped that the results of these studies will have significance for understanding the mechanism of chronic eye irritation by cigarette smoke as well as for the prevention of smoking.

Introduction

Several surveys have found the subjective sensation of eye irritation to be the most commonly reported symptom associated with cigarette smoke exposure (Speer 1968, 1971, Weber et al 1976, Weber-Tschoop et al 1976, Shepard 1978). The ocular irritant effect of cigarette smoke, however, has been the subject of relatively little investigation. Available data strongly suggests that there may be significant physiological change in human eyes chronically exposed to cigarette smoke. For example, several exposure studies have shown adaptation in the cigarette smoke-induced ocular sensation of "unpleasantness", with the threshold increasing from 4.7mg./m^3 of "smoke particulates" for non-smokers to 9mg./m^3 for smokers (Shephard 1982). Other exposure studies have shown a similar pattern of adaptation in time of onset of subjective ocular symptoms, with non-smokers reporting the onset of smoke-induced ocular irritation much earlier than heavy smokers. Further evidence of ocular function alterations from cigarette smoke exposure comes from studies showing a decreased tear film breakup-time in smokers (Basu et al 1978), as well as ultrastructural changes in the conjunctiva including loss of microvilli (Waheed and Basu 1970,

Basur and Basu 1980).

Unfortunately, none of these studies of cigarette smoke-induced ocular changes can be considered conclusive. To a greater or lesser extent, all suffer from major methodological problems, including 1. the large inherent variability of subjective human sensory responses, 2. the confounding influence of other, uncontrolled sensory imputs (taste, smell), and 3. the lack of standardization of experimental methodology.

Furthermore, no study has yet addressed the fundamentally important question of whether the chronic, irritant-induced ocular changes noted above are actually harmful--whether they are associated with any recognizable ocular disease or disability--and routine hospital data (HAA and HIPE) from the General Household Survey (1976) are not helpful in testing this hypothesis. This final consideration is of importance because, given the great concern individuals have for their eyes and eyesight preservation, any documented relationship between cigarette smoking and eye disease might have significance for smoking cessation, prevention, and health promotion.

Objectives

The objectives of this study are:

1. to systematically investigate the acute and chronic irritant effects of cigarette smoke on the human eye,

2. to determine whether chronic cigarette smoke exposure may be associated with chronic external eye disease,

3. to aid in developing recommendations for the prevention of eye disease.

To fulfill these objectives, the investigation will attempt to answer the following questions:

1. Is cigarette smoke an ocular irritant? Does exposure of the eye to normal concentrations of mainstream or sidestream cigarette smoke produce an objectively measurable, consistent, and characteristic ocular irritant response?

2. If present, what are the characteristics of this cigarette smoke-induced ocular irritant response? What is the response's speed of onset, duration, relationship to smoke-constituent dose or concentration, and relationship to the ocular irritant response already determined for other known irritant

gases and vapours (Douglas 1975, Coe and Douglas 1980)?

3. What component of cigarette smoke is irritant? Cigarette smoke is a complex mixture of particulates and known irritant gases such as formaldehyde and acrolein (Ayer and Yeager 1982). Is one constituent of the smoke primarily responsible for the ocular irritation, or do all elements act synergistically to produce the irritant response?

4. Does chronic exposure to cigarette smoke alter the ocular irritant response? Is the ocular response to cigarette smoke different in non-smokers as compared to "light", "heavy", or ex-smokers, adjusted for age and years of smoke exposure?

5. Are the ocular irrtant responses to "active" and "passive" smoking the same? Does the pulmonary and nasopharyngeal exposure to mainstream smoke in "active" smoking in any way alter the ocular irritant response determined for direct ocular exposure only?

6. Can chronic eye irritation by cigarette smoke be a factor in the development of ocular disease? Do ocular abnormalities, particularly of the ocular adnexa and anterior segment (the areas most directly exposed to the irritant effects of cigarette smoke) occur more frequently in individuals with chronic, heavy, cigarette smoke exposure.

Subjects and Methods

This investigation will have two major components, an experimental, laboratory-based study of the cigarette smoke-induced ocular irritant response, and a case-control study of the relationship between cigarette smoke exposure and abnormalities of the external eye.

1. Laboratory Investigation

The subjects for this study will be both males and females ranging in age from twenty to sixty years. Three groups of twenty subjects will participate in each phase of this investigation: the first group will consist of subjects age twenty to thirty years, the second, subjects age thirty to forty-five years, and the third, subjects over forty-five years old.

All subjects will be in good general health and, specifically, will be free of any eye disease that could interfere with the response being measured or measurement methods. The number of eye conditions that could interfere with tearing or tear measurement are relatively few: swelling, inflammation, or exudative conditions, and conditions associated with increased or decreased tearing. Because such conditions would be apparent, they should not require specialized ophthalmologic diagnosis. In lieu of an ophthalmo-

logical examination, the following questions will be answered by all subjects prior to entry into the study:

1. Have you ever had (or been told by a physician that you had) an eye disease other than a stye, or acute eye infection that resolved within one week?

2. Have you consulted an eye specialist in the past year for any cause other than those relating to eye glasses? What was the reason?

3. Have you had a "common cold", "allergy", or acute eye infection in the past two weeks?

Affirmative ("yes") responses to any of these questions would necessitate an ophthalmological examination before inclusion in the study.

Prior to participation, subjects will complete a questionnaire (Appendix B) detailing their smoking habits and exposure, age, sex, and occupation. A complete explanation of all procedures will then be given and the tearing measurement method demonstrated. Informed consent will be obtained, and the signed consent slip will be kept on file in the Department of Occupational Health. Because human subjects will be used in this study, approval by the School of Hygiene's Committee for the Control of Clinical Investigations and Experiments on Humans must be obtained. However, no difficulty is anticipated in obtaining this approval because the

method development studies using sulphur dioxide were approved in 1980.

For this study, both mainstream cigarette smoke (smoke from the proximal end of the cigarette) and sidestream smoke (the smoke plume from the distal end of the cigarette and smoke penetrating the cigarette wrapping) will be generated by a modification of the method of Carson et al (1965). Briefly summarized, "low tar" filtered cigarettes, obtained commercially, will be selected on the basis of equal weight and stored at constant temperature and humidity. For smoke generation, the cigarette will be mounted vertically in a smoking chimney housed in a fume cabinet and connected directly to the gas dilution and delivery apparatus that has already been extensively described (Douglas 1975, Douglas and Coe 1981). A reciprocating pump will produce the equivalent of one "puff" per thirty seconds with the air volume regulated to yield a burning rate of approximately one centimeter per minute. For mainstream smoke studies the smoke will be diluted with room air--a standard air:smoke dilution of 40:1 will be used in initial studies (Carson et al 1965) and, in dose-response studies, dilutions of 20:1 and 80:1 will also be studied. For sidestream smoke studies, the smoke plume exiting from the chimney top will not be diluted.

The ocular irritant response to be measured in this study is tearing (see Appendix A). In previous studies of the effect of irritant gases on the human eye, the ocular tearing response has been characterized and, within the specific limits of laboratory conditions (temperature, wind, light, time of day), has proved to be a reliable indicator of ocular irritation (Coe and Douglas 1980, 1984). In the present study, tearing will be objectively measured by the modified Schirmer test method (Coe and Douglas 1980, Douglas and Coe 1981).

In a typical exposure, after the occupational and smoking history questionnaire has been completed and control measurements made, subjects will put on the exposure goggles, apply a nose clip, and then close their eyes. Mainstream or sidestream smoke will enter the goggles for fifteen seconds, the subjects will then open their eyes and, for the remainder of the exposure period, smoke will flow into the goggles at 0.25 litres per minute, a rate previously shown to be non-irritant (Coe and Douglas 1980). Tearing measurements will be made immediately after the exposure period, and at five and fifteen minutes post-exposure. Including pre-exposure measurements, the entire duration of each experimental session will be approximately thirty minutes.

Whenever possible, a doubly-masked experimental design will be employed to insure, in so far as possible, the limitation of observer or subject-induced bias. However, in a number of the proposed determinations, the information being sought is simply whether or not a response occurs following smoke exposure. Because of the necessity of using freshly generated smoke as directly as possible, it would be difficult to make such determinations entirely masked as the variable altered (cigarette smoke or a "blank" dose of room air) would necessarially be apparent to the subject or experimenter. In these instances, the quasi-experimental nature of the study will simply be noted and the possibility of bias considered in the discussion.

In all phases of this study, subjects will serve as their own controls. This design is necessitated by existing research on human tearing that has shown significant variability in tearing levels for a given subject measured on different days, or even different hours of the day (Pinschmidt 1970, Feldman and Wood 1979, Lamberts et al 1979, Coe and Douglas 1980). Because of this great range of non-irritated ("baseline") tearing, it has never been possible to narrowly define a universal "normal" tearing level. Consequently, the comparison of a subject's tearing to that of an independent control is not possible. However,

studies of the measuring method used in this investigation have shown that, for any given subject under laboratory conditions, the non-irritated tearing level remains relatively constant over at least a forty-five minute period. Because each experimental session requires only thirty minutes, the comparison of each subject's pre- and post-irritation tearing levels should be acceptable.

II. Case-Control Studies

The "case" population for this study will be individuals visiting an ophthalmology out-patient department who 1. have been diagnosed as having a disease of the external portion of the eye included in Table 1, 2. give written consent for inclusion in the study, 3. agree to complete the smoking habit and occupational status questionnaire administered by a research assistant, and 4. agree to give a breath sample for carbon monoxide analysis. For this pilot study, sixty cases (thirty males and thirty females) will be collected. Because most of the eye disorders listed in Table 1 become more common with increasing age, and because the hypothesis is that chronic cigarette smoke exposure is associated with chronic eye disease, cases collected will range in age from forty to sixty years.

The proposed study will have two separate control groups. The first control group will consist of sixty out-patients with squint or accomodation problems. Like the cases, controls will be required to give consent for inclusion in the study, give breath samples for carbon monoxide analysis, and complete the smoking and occupational history questionnaire. Controls will be matched to cases by age and sex.

The second control group will consist of sixty out-patients with diabetic retinopathy. This ophthalmological diagnosis was chosen for a control group because it has recently been shown to be unrelated to cigarette smoking (Klein et al 1983), and is of sufficient severity to eliminate recall bias; these subjects cannot be used as the sole control group because diabetes can affect the autonomic functioning of the eye (Smith and Smith 1983) and, itself, may produce lacrimal abnormalities. As with the case subjects, the individuals in this control group will give consent for inclusion in the study, give breath samples for carbon monoxide, and complete the smoking and occupational history questionnaire. Subjects with diabetic retinopathy will also be matched to cases by age and sex.

The reliability of subjects' reports of their current smoking habits will be verified by using Ecolyzer measurement of carbon monoxide in exhaled air

samples (Hawkins et al 1975).

TABLE 1

Diagnoses For Inclusion Of Cases In

Case-Control Study

(I.C.D. Diagnoses 370.-375.)

Conjunctiva

- keratoconjunctivitis
- chronic conjunctivitis
- keratoconjunctivitis sicca
- conjunctival tumors:
  - papilloma
  - epithelioma

Cornea

- keratitis-various etiology
- corneal dystrophies

Sclera

- scleritis
- episcleritis

Eyelids

- ectropion
- blepharitis
- eyelid tumors:
  - papilloma
  - basal/squameous cell carcinomas

Lacrimal Apparatus

- non-congenital epiphoria
- dacryocystitis (in adults)

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APPENDIX A

Tears, Tearing as an Ocular Irritant Response, and Tearing Measurement Techniques

The human tear film is a complex structure divided into three layers (Holly and Lemp 1981): 1. an outer, lipid layer, approximately 0.1 μm. thick, derived from the Zeiss, Moll, and Meibomian glands of the eyelids; 2. a large, central aqueous layer containing a variety of inorganic salts, glucose, surface active agents, and enzymes, approximately 7 μm. thick, derived from the main and accessory lacrimal glands; 3. an inner, mucus layer, approximately 0.05 μm. thick, derived primarily from the conjunctival goblet cells. Under normal circumstances, tear flow is mainly in the menisci along the borders of both lids (lacrimal "rivers") towards the openings of the lacrimal ducts at the nasal borders of the lids. The tears in the menisci are then spread across the surface of the eye by each blink. Control of the aqueous layer (and, thus, of the thickness of the tear film) is a function of the facial (VII) nerve, while the mechanism controlling the glands responsible for the lipid and mucus layers is unknown.

The "normal" tear volume is currently believed to be 7.0 ± 2 μl. Of this total volume, the pre-

corneal tear film contains approximately 1 µl., the menisci 3 µl., and the upper and lower fornices 3 µl. (Mishima et al 1966). Although subject to considerable variation, the unstimulated tear production rate is approximately 1.2 µl./min., with a range from 0.5 to 2.2 µl./min. (Mishima et al 1966). Any form of eye irritation, however, can produce an almost immediate increase in tearing of more than one hundred percent (Berta 1983).

In vivo human ocular responses to irritant gases and vapours have been the subject of relatively little investigation. Much of what is known about the ocular irritant response comes from animal studies but, because of significant differences in ocular structure and physiology, this data can only be applied to human beings with great caution. However, recent studies of tearing in response to irritant gases in volunteer subjects have provided in vivo human data that at least allows preliminary formulation of the mechanism of the ocular irritant response (Coe and Douglas 1980, 1984). In these studies, the ocular irritant response has been characterized as a true neural reflex reaction that is rapid in onset following stimulation, quick to resolve following removal of stimuli, and causes an equally rapid response in the opposite, unstimulated eye. Chemoreceptors for the response are located in the

central cornea, and are functionally differentiable from pain, touch, and beta-adrenergic receptors. Finally, the afferent pathway for the irritant response is in the trigeminal nerve, with central integration possibly occurring in the main trigeminal sensory nucleus.

Several methods are available to study tear production and the characteristics of the tear film--the number, perhaps, reflecting dissatisfaction with each individual technique.

The most commonly employed method for studying tearing is the Schirmer test. In this procedure, a standard filter paper collecting strip is placed over the lower lid into the inferior fornix at the lateral third of the eye, and left to absorb tears for five minutes. The amount of tear production is determined by measuring the length of strip wetting in millimeters.

Unfortunately, as has repeatedly been shown (Wright and Meger 1962, Van Bijsterveld 1969, Pinschmidt 1970, Feldman and Wood 1979, Lamberts et al 1979), the results of the Schirmer test often are not reproducable. Of equal importance for the current study is that the Schirmer test, itself, is irritating because the collecting strip physically stimulates the cilia and lid margins. Because of this, the test is

not a useful technique for making non-irritated, "baseline" measurements of tear production, or for studies determining the effects of exogenous irritants on tear production.

Most measurements of normal tear production and volume come from studies using slit-lamp fluorophotometry (Mishima et al 1966, Jordan and Baum 1980). However, this method also has drawbacks in that it requires an extensively modified slit-lamp and the use of fluorescein--a substance also known to be an irritant (Mishima et al 1966, Lingelbach and Haberich 1982).

Tear film break-up time is the measured period between a complete blink and the appearance of the first randomly distributed "dry spot" in the tear film (Holly and Lemp 1980). Abnormal break-up times (less than ten seconds) reflect tear film instability, usually due to changes in the tear film mucus layer. Such abnormalities, however, have never been related to exogenous ocular irritants. In addition, this test also requires the use fluorescein and, in practice, its results have been shown to be quite variable (Vanley et al 1977, Stodtmeister et al 1983).

The tearing measurement technique to be employed in the proposed study is derived from the Schirmer test (Coe and Douglas 1980). However, significant changes

have been made in filter paper strip size, location of tear sampling, sampling duration, and method of analysis. In this modified Schirmer technqiue, small strips (1/8 x 1/4") of filter paper are placed under the caruncle for fifteen seconds. The quantity of tears collected is determined by weighing the strip in a closed, pre-weighed chamber. This technique offers the advantage of minimal irritation: there is little contact between the collecting strip and cilia or lid margins, and tears are sampled from one of the least sensitive areas of the eye (Norn 1973). To date, the measuring method has been used successfully in several studies of the ocular irritant response, giving results consistent with those from more complicated techniques.

APPENDIX B

Cigarette Smoking and Occupational History Questionnaire

(Note: This questionnaire is presented here in draft form. In practice, it would be printed with appropriate scoring columns, occupation codes, etc.)

General Information

To All

1. Age

2. Sex

Occupational History

3. List occupations (and years at these occupations)

Smoking History

4. Have you ever regularly smoked a cigarette, cigar, or pipe?

   Y = 1 (go to 5)
   N = 2

To All Who Have Ever Smoked

5. Do you smoke cigarettes regularly now?

   Y = 1 (go to 6)
   N = 2 (go to 7)

6. How many cigarettes do you regularly smoke a day?

To Ex-Smokers

7. Have you ever smoked regularly?

Y = 1 (go to 8 and 9)
N = 2

8. If yes, how many cigarettes did you regularly smoke a day?

9. If yes, for how many years?

To All Who Have Ever Smoked Regularly

10. Do you smoke a pipe regularly now?

Y = 1 (go to 11 and 12)
N = 2 (go to 14)

To Current Pipe Smokers

11. If yes, how many pipes a day?

12. If yes, for how many years have you regularly smoked a pipe?

13. If no, have you ever smoked a pipe regularly?

Y = 1 (go to 14 and 15)
N = 2

To Ex-Pipe Smokers

14. How many pipes did you regularly smoke a day?

15. For how many years did you smoke pipes regularly?

To All Who Have Ever Smoked

16. Do you smoke cigars regularly now?

Y = 1 (go to 17 and 18)
N = 2

To Current Cigar Smokers

17. How many cigars do you regularly smoke a day?

18. How many years have you regularly smoked cigars?

19. If no, did you ever smoke cigars regularly?

Y = 1 (go to 20 and 21)
N = 2

To Ex-Cigar Smokers

20. How many cigars did you regularly smoke a day?

21. For how many years did you regularly smoke cigars?