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CONSCIOUSNESS, NEURONS, AND LAUGHING GAS

by

DODY MICHELSON ORENDURFF

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE
in
PSYCHOLOGY

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1979

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TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

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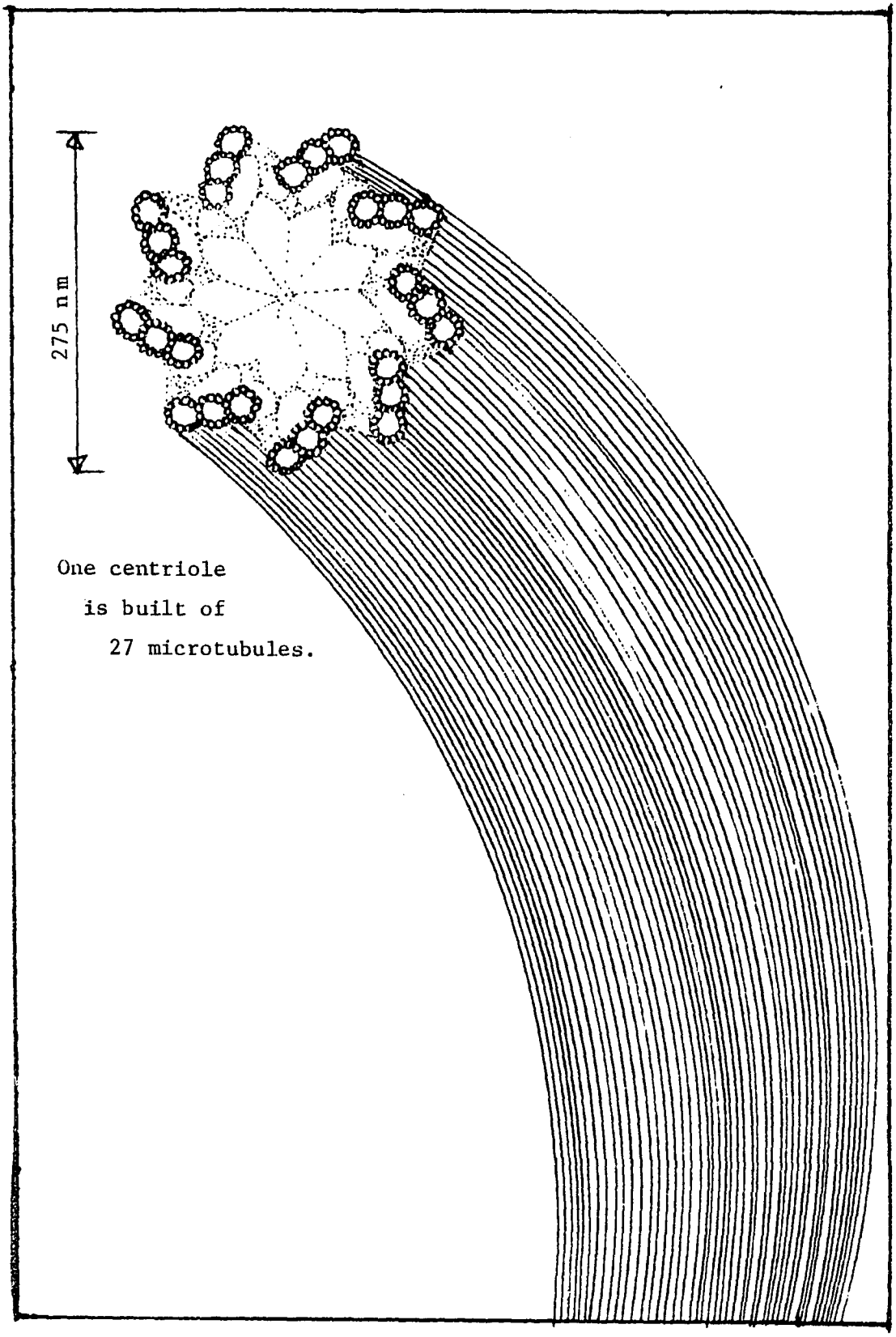
AN ABSTRACT OF THE THESIS OF Dody Michaelson Orendurff for the Master of Science in Psychology presented August 1, 1979.

Title: Consciousness, Neurons, and Laughing Gas.

Psychological and physiological effects of nitrous oxide resemble those of eight other drug categories. Lipid solubility or hydrate microcrystal theories correlate behavioral measures with measurable parameters of the molecule N_2O. N_2O, a spindle poison, halts mitosis in metaphase, producing widespread physiological consequences. N_2O affects the microtubules of the spindle in a number of specific ways. Microtubules are utilized in other parts of eukaryotic cells, in a wide variety of functions. In neurons, microtubules build and maintain dendritic sensory processes.

Since microtubules are built of two dissimilar proteins, constantly assemble and disassemble, and maintain a more negative interior potential, they would be responsive to changes in summed post-synaptic dendritic potential.

Microtubules respond to N_2O with a loss of communication between subcellular components, and between cells. Chromosomes, proteins, and ATP are no longer transported efficiently. Such fundamental changes might explain nitrous oxide's effects in "potentiating" other drugs, and upon perception and memory.



One centriole
is built of
27 microtubules.

This Thesis

is

Dedicated

to

My Beloved Children

Karen

Rachel

Michael

Rebecca

Deborah

Malcolm

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CHAPTER I

INTRODUCTION

This thesis is a review of the literature on nitrous oxide, laughing gas, with the intention of developing a new model of how nitrous oxide produces effects upon the experience, consciousness and behavior of humans. I chose to study nitrous oxide (1) because of its wide use, both in surgery and in the dentist's office; (2) because of its chemical simplicity and biologically inert qualities; (3) because it produces remarkable subjective experiences and physiological effects; and (4) because, although many theories exist, its mechanism of action has not as yet been determined.

Present day experimental psychology emphasizes two major fields of study: the behavior of organisms, and the physiological processes underlying that behavior. The subjective experiences of humans in an experimental situation are frequently ignored, while their reaction times or blood pressures are carefully monitored. This thesis will emphasize human experience under nitrous oxide, as well as effects of nitrous oxide upon cells, neurons, and parts of living neurons. In emphasizing both experience and neuronal functioning, I am rejecting dualism,

the separation of mind from brain and body, and I am rejecting mechanism, the view that the brain and body are machines. I hold the view of the identity of consciousness and matter, of the continuum of the development of consciousness from the earliest species of creatures to the latest. "Wherever there are organs of perception, however crude, there must be a perceiving consciousness, however dim" (Firsoff, 1967, p. 47). I shall begin by reviewing historical definitions and criteria for consciousness.

A modern definition of consciousness is that state of being which is characterized by sensation, emotion, and thought. A behavioral definition of consciousness or awareness considers the question: can the subject discriminate between stimuli presented? Nitrous oxide is a drug that affects perception, discrimination between stimuli, motor coordination, motivation, cognition, awareness, emotion, memory, one's sense of identity, and one's consciousness of inhabiting a body in a particular time and place. For these reasons it seems to me to be a drug that a psychologist might well spend time investigating. Since the study of psychology also includes the study of the physiological functioning of the brain and the neuron, it will not be outside the realm of psychology for me to investigate the effects of nitrous oxide upon neurons, including their subcellular protein systems.

As a method of approach to understanding modern definitions of states or degrees of consciousness, I shall review stages of anesthesia (a state of unconsciousness); analgesia (a state of feeling no pain); amnesia (a state of loss of memory); euphoria (a state of pleasure, joy, or bliss); and psychedelia (a state of altered perception). It is instructive to compare the subjective reports of persons who have breathed nitrous oxide (Roget, 1799; Davy, 1799; Blood, 1879; James, 1899) with the subjective report of a person who has used opium (de Quincey, 1821).

Having reviewed historical and current definitions of consciousness, I shall review human behavioral studies of the effects of nitrous oxide. These experiential and behavioral effects of N_2O will be followed by a review of physiological effects of N_2O, both in vivo and in vitro. I shall start that section by reviewing the toxicity of nitrous oxide and some problems in the medical use of N_2O. I am collecting effects of N_2O from a number of different fields of study and from a wide variety of journals. Nitrous oxide affects the immune response as well as the growth and development of embryos. Anesthesiologists have noted a rise in plasma norepinephrine levels as nitrous oxide enters the system. Geneticists have noted that nitrous oxide acts like a "spindle poison," halting mitosis in metaphase. Nitrous oxide has caused spontaneous abortions, miscarriages, and birth defects

in the children of both female and male anesthesiologists, dentists, and nurses who are exposed to trace levels of N_2O while they work.

After I have reviewed physiological effects of nitrous oxide in vivo, I shall review effects of N_2O upon cells, including neurons, in vitro. As a psychologist, I am searching for the links that might join a particular experiential or behavioral effect with a physiological effect of N_2O upon the neuron. I shall review some past hypotheses about the mechanism of action of nitrous oxide, beginning with the hypothesis that N_2O interferes with the transport or use of oxygen.

I shall review theories of anesthesia. Nitrous oxide is not an anesthetic, but it is given in large quantities with the actual anesthetic. It is included with anesthetics or inert gases when investigators attempt to develop a theory of anesthesia. I shall review the oldest theory, that of lipid solubility, as well as a theory by Linus Pauling on hydrate microcrystals. I shall review theories that relate anesthesia to polarizability, surface film affinity, mole volume, and dipole moment. Nitrous oxide is considered in these theories. I shall review cation permeability theories and pressure reversal of anesthesia.

Nitrous oxide is an analgesic, comparable in potency to morphine. I shall review theories of analgesia and

our endogenous opiates. In my search to understand how nitrous oxide produces analgesia, I shall review brain distribution of opiate receptors to brain transmitters. I shall review brain electrical stimulation, which may involve the release of endogenous molecules similar to the opiates, as well as experiments that seem to relate N_2O to this same phenomenon, the release of endogenous "opiates."

Since nitrous oxide has been shown to have an effect upon cell division, halting the process of mitosis in metaphase, I shall review the development of the neuron, with an emphasis on the role played by microtubules, both in cell division, and in the lifelong growth, development, and moment-to-moment functioning of our neurons.

I have mentioned how nitrous oxide affects the immune response, causing a sudden drop in the red blood cell production, leading to anemia, and a sudden drop in white blood cell production, leading to illness and death. I have mentioned the teratogenic activity of nitrous oxide, that it produces death of embryos, or birth defects, both in experimental animals and in humans whose jobs lead to occupational exposure to trace levels of N_2O. I shall review physiological experimental studies on the effects of N_2O upon the process of mitosis. Humphrey Davy (1799) was the first to notice that plants will not grow, seeds will not germinate, in an atmosphere containing nitrous

oxide. Later research has shown that N_2O, like other "spindle poisons," halts mitosis in metaphase. If N_2O treatment lasts for 4 hours, cells can recover and proceed through mitosis. If N_2O lasts for 12 or 16 hours, cells cannot recover. Abnormal cells, with two nuclei, occur.

Having reviewed the essential and versatile functions of microtubules in cells, especially neurons, I shall review how nitrous oxide blocks mitosis, how it affects microtubules. These effects can be seen with an electron microscope.

I shall then present a theoretical proposal for the mechanism of action of nitrous oxide.

CHAPTER II

HISTORICAL DEFINITIONS OF CONSCIOUSNESS

Over the centuries philosophers, physiologists and psychologists have wrestled with the problems of defining consciousness, setting criteria for consciousness, and measuring various aspects of consciousness.

Aristotle (384-322 B.C.) was the first to view the mind of the newborn infant as a tabula rasa. He numbered our senses as five, and mentioned the complex nature of our sense of touch. He noted that memory classifies objects according to their similarity, their contrast, or their contiguity. Although his philosophy foreshadowed materialism by dividing things into form or matter, he did not embrace determinism, holding that mind is one, and mind is free.

MECHANISTIC DUALISM

René Descartes (1596-1650) accepted this view of the mind as one and free. Descartes, a mathematician, sought for evidence and proof, by a method of doubting everything around him. He found that he could not doubt the existence of the perception, the doubt, or the thought. From his he concluded that the perceiver, the doubter,

the thinker himself, also existed. (Santayana (1937) has pointed out that, at the moment of perception, we are unaware of ourselves as perceiver, and hence he doubts even Descartes' "Cogito, ergo sum.") Descartes, sure of the existence of his own mind only, then asked if matter exists. Material things include our own brains, bodies, other humans, animals, all other nature, all of the material universe. He concluded that all these things exist since God exists, and God would not fool us. Animals, having no minds, are automata. Even the human body is mechanically controlled, although it interacts with the mind. Reflex action, as well as the circulation of the blood, were examples of our machine-like bodies. In his separation of mind from brain, Descartes was the first dualist in psychology, and a number of Cartesian materialists have followed his ideas to even more extreme conclusions.

Julien de La Mettrie (1709-1751) extended Descartes' mechanistic views of the brain and body to include determinism and predestination. La Mettrie came to believe that "thought is after all nothing but the result of the mechanical action of the brain and the nervous system" (Boring, p. 213). He can thus be seen as the first behaviorist, the first "objective" psychologist.

Immanuel Kant (1724-1804) re-established Cartesian dualism. Kant viewed the conception of space as a native perception, a given physical property of the nervous

system. Müller, Hering, Mach and Stumpf followed Kant's perceptions on the nature of the physical brain. Georg Müller (1850-1934) studied sensory attention methods of psychophysical measurement, constant stimuli, the psychophysics of vision and memory. Ewald Hering (1834-1918) investigated problems of visual space perception, and believed that space perception is innate. He endowed the cells of the retina with sensitivity for height, depth, and right-left position. Hering later developed his three-retinal cell, six-color theory: in this red-green are opposing responses of one type of cell; yellow-blue responses of another type of cell; and white-black for the third type of cell. Hering's temperature theory supposes warmth and cold to be sensed by similar opposing processes. Hering saw sensations as a part of consciousness.

Ernst Mach (1838-1916) investigated visual space perception, theory of hearing, the sense of time, the perception of rotation, and the functions of the semi-circular canals. Mach believed all science to be observation; sensation is the basis of this observation; introspection is justified as a method of observing conscious data. Sensations are the "given" data; there is no ego beyond these.

Carl Stumpf (1848-1936) studied the origin of space perception, and believed both color and extension to be

innate visual sensations. His most famous studies were his investigations into hearing, music, tones, tonal fusion, attention, analysis, practice and fatigue.

These early mechanists were all pursuing the study of psychophysical phenomena in human beings. There is a later group of mechanists who studied animal behavior. Lloyd Morgan (1852-1936) first stated their credo of parsimony, an attempt to interpret every behavior as the outcome of the lowest possible state of development of the animal mind. Jacques Loeb (1859-1924) was able to follow the spirit of this mechanistic belief in his work on tropisms. Loeb held as a criterion of consciousness the ability to develop an "associative memory." He had faith in physical and chemical methods as a tool for studying behavior.

The mechanistic viewpoint was extended to Thomas Beer, Albrecht Bethe (1872-1931) and J. von Uexküll, who proposed in 1899 to discard all psychological terms and to write only of receptions and reflexes. They suggested that the social insects might be robots. I. M. Sechenov (1829-1905) studied neural action, the Reflexes of the Brain (1863). He believed that "all acts of conscious or unconscious life are reflexes" (Boring, p. 635). V. M. Bekhterev (1857-1927) extended Sechenov's work to include Human Reflexology (1917), an effort to battle the methods of introspection.

Ivan Pavlov (1849-1936) was a physiologist studying the pancreas who won the Nobel Prize for this research. His technique of the conditioned reflex was a measure of anticipation. Conditioning could thus substitute for introspection, could become a language for communication between animal and scientist. This language uses only the terms of stimuli, nerve-action, and secretion. Consciousness is a superfluous concept.

John B. Watson (1878-1958) the founder of behaviorism, began his career with a thesis on Animal Education: The Psychical Development of the White Rat (1903); then came Neurological and Psychological Maturation of the White Rat and Somesthetic Sensation in the Rat in the Maze (1907). In these papers Watson still drew inferences about animal consciousness from his research on animal behavior. However, in 1913 Watson "announced that introspection must not be employed and that only motor (and glandular) activities must be discovered" (Boring, p. 565). Watson thus surpassed even Lloyd Morgan in removing consciousness from the field of study of psychology. Watson wished psychologists to ignore consciousness, so as to deal with the reliable data of behavior. Watson translated concepts of imagery, feeling and association into behavioristic terms. Verbal responses and subvocal thinking or vocimotor behavior were the counterparts of imagery and thinking. Feeling turned out to be tumescence or

detumescence. Association was, of course, the conditioned reflex. Behaviorism could be used on infants; "Little Albert" was thus frightened. It could be used in advertising, Watson's field of interest after he resigned from Hopkins in 1920. Watson's methods of controlling us through advertising are still with us today in every TV commercial.

Edwin Holt (1873-1946) wrote The Concept of Consciousness (1914); he believed that only through behavior can we understand mind. Holt studied behavior as a whole, including its purpose. Behavior, the response relation, is meaning. Behaviorism is a psychology of meanings.

Edward Tolman (1886-) studied the temporal relations of meaning and imagery, writing a behavioristic definition of consciousness. It did not matter whether rats were conscious or not; their actions were purposive and objective. Tolman saw the data of consciousness as ineffable, not public, and hence not scientific data.

Karl Lashley (1890-1958) discovered the imprecision and delocalization of brain functions. He was a physiological psychologist, who felt the concept of consciousness was unnecessary. He wrote the Behavioristic Interpretation of Consciousness (1923). He attempted to discover the neural bases for learning and discrimination.

Albert Weiss (1879-1931) believed that all the phenomena of psychology could be reduced to physical-chemical terms or to social relations. Thus we do not

need the conception of consciousness.

Walter Hunter (1889-) wrote against introspection; he translated descriptions of consciousness into terms of stimulus and response.

B. F. Skinner (1904-) published The Behavior of Organisms (1938). He does not see behavior as essentially purposive, but has developed a reflexology instead.

Clark Hull (1884-1952) studied hypnosis and suggestibility, as well as conditioned reflexes and learning. He formulated a Mathematico-Deductive Theory of Rote Learning (1940) and wrote Principles of Behavior (1943).

I have briefly described the changing viewpoints of these scientists, from Descartes to Hull; I have followed the development of mechanism in psychophysical phenomena, in comparative psychology, and in behaviorism.

THE IDENTITY OF CONSCIOUSNESS AND MATTER

A different tradition of approaching the study of mind began with Wilhelm Leibnitz in 1680. This view emphasizes the identity of consciousness and matter, the continuum of the development of consciousness from the earliest species of creatures to the latest. Following this emphasis on perception and experience were Locke, Berkeley, Herbart, Weber, Fechner, Helmholtz, Wundt, James, Ebbinghaus, Darwin, Binet, and H. Jennings.

John Locke and Wilhelm Leibnitz were both influenced

by Descartes, and both drew up philosophies that opposed the mechanism of Descartes.

John Locke (1632-1704) wrote his Essay Concerning Human Understanding (1690) when he was 57 years old. Locke saw ideas as the units of mind. Ideas are logical concepts, meanings, or items of knowledge. Ideas are not innate, as Descartes believed, but come from experience. Locke thus expanded Aristotle's concept of the tabula rasa. Ideas come to us from reflection as well as from sensation.

Wilhelm Leibnitz (1646-1716) developed a far more comprehensive view of mind than did John Locke. Leibnitz believed that Nature cannot be explained in a mechanistic way. "The ultimate elements of the universe are individual centers of force; they are simple, percipient, self-active beings. The very atoms of nature are centers of force, without parts, extension or form" (Sorley, 1952, p. 886). (Here we might use the word 'quark' instead of atom.) Space and time are merely relative. Consciousness lies along a continuum, and develops. "Different species of creatures rise by insensible steps from the lowest to the most perfect form" (Sorley, 1952, p. 886).

In the human mind perception is developed into self-conscious thought. The appetite of the most primitive creatures develops into will. The spontaneity of a protozoa develops into the freedom of a human being. "The end determining the will is pleasure, and pleasure is

the sense of an increase of perfection. In love one finds joy in the happiness of another; and from love follow justice and law" (Sorley, 1952, p. 887).

George Berkeley (1685-1753) saw perception as reality, saw ideas as the one thing of which we are sure. This extreme view leads to solipsism, a doubting of the existence of anything beyond one's own immediate perceptions.

Johann Herbart (1776-1841) wrote "Psychology as Science, newly grounded on Experience, Metaphysics, and Mathematics" (1825). He believed in an empirical psychology, using the method of observation. Perceptions (or ideas) seem to differ from each other in quality; each one is individual; we do not mistake sweet, red, or hard. Perceptions also vary in intensity and in duration. Intensity, force, or clearness, may be sufficient to bring the perception (or idea) across the threshold of consciousness. The perception enters, crosses the limen (threshold), and is assimilated into memory, the previous totality of conscious ideas. In this sense memory may merely consist of recognition of a perception as familiar, or else of the sudden attention to a perception that is strange. Herbart envisaged the totality of perceptions as actively selecting for assimilation those new sense-impressions which stand out in uniqueness (differing in quality from the old) or in intensity, or in duration. The rest do not enter consciousness, and are suppressed. Herbart's

method of observation is a transition from the pure speculation of Kant, to the methods of experimentalism of Fechner, Helmholtz, and Wundt.

Ernst Weber (1795-1878) wrote Touch and Common Sensibility (1846). He saw sensation as varying in quality (pressure, temperature) and in degree. Spatial location and duration depend on the activity of the mind in interpreting the local sensations. Weber saw that the smallest perceptible difference can be expressed as a ratio: 1/40 for weights; 1/50 to 1/100 for lines; and 1/160 for tones.

Gustav Fechner (1801-1887) rejected dualism as an unreal view. Even psychophysical parallelism did not represent reality. Fechner upheld the identity hypothesis as the relation of body and mind. Only for purposes of measurement do we need to regard body (or sensation) as different from mind (or perception). Rather than following Herbart's view of a limen of excitement crossed with a stimulus of sufficient intensity, Fechner believed this to be a limen of consciousness, since there is a distinction between attention and inattention, as well as one between sleep and waking. Fechner developed three ways of measuring this limen of consciousness: the just noticeable difference, or method of limits; the method of constant stimuli; and the method of adjustment. He extended Weber's Law, $\delta R/R = k$ to Fechner's Law $S = k \log R$, where R = magnitude of the stimulus and S = magnitude of the sensation or

perception.

Hermann von Helmholtz (1821-1894) investigated almost the whole field of science, from mechanics to physiology. At 26, he read his paper On the Conservation of Force to physicists; by 30, he had invented the opthalmoscope. He studied the lens of the eye, accommodation for distance, color vision, and binocular vision. He gave a secure foundation to Thomas Young's theory of color vision, and showed the three primary colors to be red, green, and violet. He applied this theory to explain color-blindness. At 45, he wrote Physiological Optics. He studied hearing, explained the bones of the middle ear, and applied principles of sympathetic vibration to explain hearing in the cochlea. He explained tonal quality, due to the order, number and intensity of harmonics. He wrote Sensations of Tone (1862). He gave a velocity of light figure of 314,000 meters per second.

Helmholtz was an empiricist. He did not follow the doctrine of innate ideas. He believed that all knowledge is founded on experience. Kant and Hering had viewed space-perception as innate. Müller also felt space is native to the mind. Helmholtz could accept the theory of specific nerves, specific retinal cells, but he argued that the integration of these nerve energies into our whole concept of space around us is an inference of mind, built by all our past experience. Helmholtz noticed

that some double images are perceived to be single, after a period of experience. Helmholtz's theory of perception embraces the doctrine of unconscious inference. By this he means that humans who know nothing at all of geometry still come to recognize objects as near or far by the associated tension in the eye muscles. These unconscious inferences are not correctable by conscious effort, and thus are normally irresistible. Sensation occurs before the unconscious inference; perception depends on it. Imagination and memory enter into these unconscious inferences. Scientific observation depends on the unconscious inferences of the scientist.

In 1845, when Helmholtz was 24, he joined three other young physiologists, Carl Ludwig, Emil du Bois-Raymond, and Ernst Brücke in a declaration against vitalism. There are several meanings attached to this concept. One of these is the question "Can we explain the functions of a living organism with physical and chemical forces? Or do we need to bring in a different force, the vital principle?" Another slightly different meaning of vitalism lies in the question, "Are the processes of life explicable by the laws of chemistry and physics alone?" We might now ask, "Which laws? Those discovered in Helmholtz's time, or by 1845? Those discovered so far, by 1979?" The difficulty is that there are still mysteries, not yet explained in spite of our explosive growth of physical

and chemical knowledge during these years. The third meaning of the word vitalism lies in the assertion that "Life is in some part self-determining."

Helmholtz certainly did not wish to bring in a different force, the vital principle, which may be characterized by God, the soul, or pantheism. He wished to explain vision and hearing, using the laws of optics and harmony. But in his doctrine of unconscious inference, in believing that all knowledge is founded upon experience, he was offering explanations of living processes which went beyond the laws of chemistry and physics. Mind seemed to show some kind of self-determining properties.

Wilhelm Wundt (1832-1920) saw perception as different from the physiologist's sensation. He used the phrase "unconscious inference," as did Helmholtz. Wundt said, "All psychology begins with introspection" (Boring, p. 320). Introspection was analytical, an attempt to resolve an experience into its different sensations. Wundt analyzed feelings into three different dimensions: pleasant-unpleasant, calm-excitement, and strain-relaxation. These dimensions are not orthogonal, since calmness and relaxation are almost synonymous. Wundt hoped to find bodily correlates, such as pulse and breathing, for each of these six directions along his three-dimensional scheme.

Wundt defined psychology as dealing with immediate experience; the method of examining this subject is that

of introspection. Wundt was a psychophysical parallelist; the elements of phenomenal experience are the mental processes, the sensations, images, and feelings. Conscious data are interdependent. The active mind naturally grows and develops by lawful processes. Perceptual associations develop by fusion (as harmonics are fused into the complex tone) by assimilation (as optical illusions take place) and by complications (as visual images include a component of temperature or sound). Wundt differentiated between the field of consciousness and the focus (the range of attention).

Franz Brentano (1838-1917) wrote Psychology from an Empirical Standpoint (1874). He thought that an over-emphasis on the methods of experimentation led to a tendency to ignore the main issue, the interpretation of experience. Perception involves actions which possess both intention and reference. Acts include sensing, imagining, perceiving, rejecting, recalling, resolving, intending, feeling, wishing, desiring.

William James (1842-1910) studied chemistry, anatomy, medicine and physiology. In The Principles of Psychology (1890) he discusses the structure and functions of the brain, as well as the evolution of consciousness. James saw that "The self-same atoms which, chaotically dispersed made the nebula, now . . . form our brains. (p. 146) . . . If evolution is to work smoothly, consciousness

in some shape must have been present at the very origin of things" (James, p. 149).

Hermann Ebbinghaus (1850-1909) wrote his doctoral dissertation on the philosophy of the unconscious. He read Fechner's work on sensation and perception and wished to extend such scientific methodology to the study of memory and thought. Using nonsense syllables, and himself as the sole subject, he was able to study the higher mental processes. He went on to study brightness contrast, color vision, and mental testing for children.

Oswald Külpe (1862-1915) began his studies with the radical view that what can not be observed does not exist, for science. However, as his work progressed, he studied sensations, reaction time, and attention, and defined psychology as the science of the facts of experience. He continued to study memory, feeling, tonal fusion, colors, touch blends, and emotions, as well as time sense and space perception. He wrote about the will, about self-consciousness, about imageless thought, esthetics, abstraction, and association. He attempted to find what is in consciousness besides sensations and images. He studied the psychology of thought, distinguishing thoughts from thinking.

Edward Titchener (1867-1927) wanted to study sensory consciousness and the mind. He wrote Psychology of Feeling and Attention (1908), and Experimental Psychology of the

Thought Processes (1909). Titchener gave to feeling only the dimension of pleasantness or unpleasantness. He felt that sensory processes have an attribute of clearness, as well as quality and intensity. When attention shifts, it is clearness, or vividness, that is changing. Titchener was opposed to Külpe's imageless thought, although he agreed that thought may be unconscious. He felt that introspection was the method to use in psychology, and he had faith in the importance of consciousness.

In this chapter I have briefly reviewed some historical definitions of consciousness. I have not attempted to give a complete or comprehensive review. I have noted that, while the mechanistic dualists have removed the study of consciousness from the domain of psychology, there still remains a group of psychologists who take seriously the study of consciousness. There seems to be a recurring interest in the topic of consciousness, which rises afresh each decade, each year, as new students and investigators enter the field of psychology. I wish to continue this tradition of attempting to explore consciousness. As a method of exploring consciousness, one can observe what sort of altered states of consciousness are produced by different drugs. Nitrous oxide is one of the very simplest drugs, and yet it produces a wide variety of changes in states of consciousness. I shall review the different states of consciousness,

mentioning other drugs that seem to produce states similar to those of nitrous oxide. When possible, I shall describe the mechanisms of action by which these other drugs produce their effects. In the next chapter, I shall review the states of anesthesia, analgesia, amnesia, euphoria, and psychedelia.

CHAPTER III

STATES OR DEGREES OF CONSCIOUSNESS

ANESTHESIA

Responsiveness is gradually lost by a human being entering anesthesia, a state of unconsciousness. Anesthesiologists have developed behavioral definitions of the different stages of anesthesia. Nitrous oxide is not a powerful enough drug, when given only with oxygen, to cause surgical anesthesia, that state of unconsciousness in which the subject does not respond to the first slice of a scalpel across the abdomen.

One of the ways of studying consciousness is by examining the effects of anesthetics. By studying anesthesia we gain an understanding of consciousness. Different anesthetics may utilize different mechanisms of action, but anesthesiologists have developed a system for classifying the stages of anesthesia (Jenkins, 1969). Behavioral measures used to assess the depth of early stages of anesthesia include:

(1) mental performance, such as answering questions or mathematical problems;

(2) subjective reports on sensations experienced or anxiety level ("I feel the pain, but I don't care.")

(3) amnesia for recent events;

(4) electroencephalogram changes;

(5) analgesia;

(6) the disappearance of color vision;

(7) impaired cerebellar functioning (the subject may stagger);

(8) ataxia, the inability to coordinate voluntary muscle movements;

(9) nystigmus, sudden eye movements that go one way then correct back in a series of jerks;

(10) inability to focus eyes.

All these changes take place during what is termed Stage I of anesthesia. Electroencephalograms show waves of 50 to 100 microvolts in amplitude, moving at a fast frequency of 20 to 25 cycles per second. Normal alpha rhythm variability disappears.

Stage II, a period of delerium and excitement, is most clearly observable, at the onset, with the electroencephalogram. There is a sudden appearance of wide, slow swings, with an amplitude of 200 to 300 microvolts, and a frequency of 2 to 8 cycles per second. These waves are rhythmic and steady. During Stage II, the patient may laugh, shout, sing, and thrash about. Amnesia and analgesia increase. He does not appear conscious of what he is doing. The jaw becomes set, skeletal muscle tone

increases, and breathing is irregular. The patient struggles. Muscle movements are uninhibited. Reflexes are exaggerated. The pupils dilate. Ocular movements are erratic. There may be retching, vomiting, urination, and defecation. There is hypertension and tachycardia. When there is a sudden increase in anesthetic vapor, there is reflex swallowing and respiratory arrest. All this is exhausting for the patient.

Stage III starts with the beginning of surgical anesthesia. EEG waves show a sudden loss of rhythmicity. A complex of slow waves with superimposed faster discharges appears. There are roving movements of the eyeballs. Respiration is entirely automatic; breathing is regular because psychic influences are absent and voluntary pathways are interrupted. There is no eyelid reflex, no blinking. An arm that is lifted and released falls flail-like.

As surgical anesthesia deepens, a new change can be observed on the electroencephalogram. The amplitude of the waves definitely falls to 150 microvolts; frequency falls to 2 to 4 cycles per second. Periods of inactivity occur, during which amplitude falls to less than 20 microvolts. These burst suppression periods last no longer than 3 seconds. The eyeballs are fixed, and the pupils dilate. When there is a sudden increase in the concentration of the gas or vapor, there is no reflex swallowing

and no respiratory arrest. There is a delayed thoracic inspiratory effort.

At a still deeper level of surgical anesthesia, EEG burst suppression periods last longer, from 3 to 10 seconds. The pupils show a light reflex. There is a beginning paralysis of intercostal muscles, and a loss of muscle tone.

At the deepest level of surgical anesthesia, EEG burst suppression periods last longer than 10 seconds, and the amplitude of the waves between suppression periods has fallen to about 70 microvolts. Pupils no longer show a light reflex, and intercostal muscles are completely paralyzed.

There is a Stage IV of anesthesia, but it is described only so that it can be avoided. The electroencephalogram shows essentially no measurable waves. All respiratory effort ceases, bringing about respiratory paralysis. The circulation fails. There is vasomotor collapse. Death follows.

Surgical anesthesia, termed Stage III, would certainly seem to be a state of unconsciousness. However, there have been a number of articles that describe awareness during surgery (Parkhouse, 1960; Rosen, 1959; Pearson, 1961; McIntire, 1966; Terrell, 1969). Memories seem to be stored, during the apparent unconsciousness of surgical anesthesia, of remarks made by surgeons. These

memories have been recalled during hypnosis a month later.

ANALGESIA

Analgesia, freedom from pain, differs from anesthesia, a state of unconsciousness. Pain is difficult to define or to describe. Some humans are born with a congenital insensitivity to pain (McMurray, 1950; Baxter, 1960). Familiar weak analgesics are aspirin and tylenol; xylocaine is injected into jaw neurons in dentistry; cocaine is an excellent local analgesic for work on the eye; codeine, perkodan, morphine and heroin, all opiates, are well known for both analgesia and euphoria. Demerol is a substitute for morphine; methadone is a substitute for heroin. Fentanyl is the strongest analgesic, 80 times the strength of morphine. Nitrous oxide is as good an analgesic as morphine.

AMNESIA

Amnesia begins as a "dazed" state in which the person reacts somewhat automatically to the environment, guiding his movements by external stimuli, but not aware of what he is doing, and not aware of the fact that he has lost his personal identity.

This is followed by a "bewildered" state in which there is loss of personal identity, and the individual is aware of the loss and troubled by it. This is followed

in turn by a return to "normal" in which personal identity is restored, even though there may still be some gaps in memory, particularly for the events of the first stage.

Nitrous oxide sometimes produces an amnesia of a few hours, in which humans appear normal, can drive a car accurately, yet cannot find the way home, cannot recognize their own street. This amnesia is puzzling because under nitrous oxide it appears so early in the progession, of descent through Stages I and II of anesthesia. Under nitrous oxide given in a dental office, at a level of 25% N_2O up to perhaps 45% N_2O, the patient can answer questions, can report that anxiety is lowered, and that analgesia is present. The more extreme changes of Stage I, loss of color vision, impaired cerebellar functioning, ataxia, nystigmus, and the inability to focus the eyes, all these seem to be absent under these light doses of N_2O. When the drilling is finished, and the nitrous oxide turned off, the patient appears to recover a completely normal state of consciousness. He appears normal to the dentist, the hygienist, the secretary. He exits to drive his car home. The task of driving is performed normally. Reflexes such as brake reaction time are unimpaired.

Nevertheless, about one patient in 20 experiences a lingering amnesia that may last several hours. He reacts automatically while driving, but he is not aware of where he is going, and may drive for an hour in the wrong

direction from his home. He is not yet aware that he is lost. This is followed by the bewildered state in which he realizes that he is lost, and cannot recognize the streets. He is aware of his temporary amnesia and is troubled by it. Slowly his orientation to places and streets is restored, and he returns to his home, but he may then still have some gaps in memory for his thoughts while he was "lost."

Besides the dimensions of awareness, anesthesia, analgesia, and amnesia, there are a number of other altered states of awareness. The most obvious and universal altered states of consciousness are those of sleep and dreaming. Other altered states include day-dreaming, meditation, the state of hypnosis, hypnogogic states, euphoria, hallucinations, delerium, and the neuroleptic state.

EUPHORIA

Nitrous oxide is one of a large class of consciousness altering drugs. Its excellent analgesic properties were recognized in 1799 by its discoverer, Humphrey Davy. Nitrous oxide also produces euphoria, a sense of bliss, of being past caring, a "pleasurable delerium . . . a highly pleasurable thrilling in all the muscles . . . exhilaration . . . sublime emotions . . . excitement equal in duration and superior in intensity to that occasioned

by high intoxication from opium or alcohol . . . a pleasurable trace" (Davy, 1799). The euphoria produced by nitrous oxide has been described by Humphrey Davy, Peter Mark Roget, Benjamin Blood, and William James (see Appendix).

PSYCHEDELIA

There are aspects of the experiences and perceptions of these writers which suggest that nitrous oxide produces not only euphoria but also a state of consciousness which is best described as the psychedelic state. The person who breathes nitrous oxide often perceives and comprehends the world in a novel way. The psychedelic state could be produced by a change in the direct impressions of the senses, but it probably also involves a change in how we interpret those sensory impressions. Such a change in perception is a different state of consciousness from those discussed so far: anesthesia, analgesia, amnesia, and euphoria. The psychedelic state may occur with one of the other states, or it may occur without them. Such a state of consciousness may include vivid images in any sense modality, sometimes termed hallucinations, or it may not include such effects. Nitrous oxide occasionally produces visual effects; it often produces effects on auditory perceptions; the frequent sensation of "floating" resembles an hallucination of touch.

Because of its analgesic properties, nitrous oxide

is one of the most widely used drugs. Surgical operations typically use very small amounts of the actual anesthetic, halothane, methoxyflurane, or sodium pentothal. Oxygen is provided, in larger percentages than exist in air. The remainder of the gas inhaled is nitrous oxide, 60% or 70% of the mixture. Humans absorb a liter of N_2O every minute for thirty minutes before their tissues are saturated with nitrous oxide (Dripps, 1972, p. 127). Nitrous oxide acts as an analgesic, and it potentiates the strong anesthetics, so that lesser amounts of these more dangerous drugs are needed (Dripps, 1972, p. 121). In spite of this widespread use, the mechanism of action of nitrous oxide is not understood. I chose to explore the effects of N_2O, both in living creatures and in laboratory tissue cultures, because of its wide use, its chemical simplicity, and its unknown mechanism of action, as well as because it alters consciousness in complex and mysterious ways.

I have reviewed the different states of consciousness produced by nitrous oxide. I shall, in the next chapter, review human behavioral studies of the effects of nitrous oxide and, in the following chapter, physiological effects of nitrous oxide.

CHAPTER IV

HUMAN BEHAVIORAL STUDIES OF THE EFFECTS OF NITROUS OXIDE

Human behavioral studies on nitrous oxide have been performed by Davidson, 1924; McKinney, 1932; Marshall, 1937, 1938; W. P. Chapman et al., 1943; Wilson et al., 1950; Steinberg, 1954, 1955, 1956, 1957, 1961; Parkhouse et al., 1960; Robson et al., 1960; Frankenhaeuser and Beckman, 1961; Frankenhaeuser and Järpe, 1962; Frankenhaeuser et al., 1963a; Frankenhaeuser, 1963b; Lader and Norris, 1969; Jarvis and Lader, 1971; Biersner, 1972; C. R. Chapman et al., 1973; Garfield et al., 1975; and Bradley and Dickson, 1976. The earlier studies mention the subjective effects reported by the subjects, such as parasthesia, difficulty of concentrating, a subsequent prolonged feeling of depression, slight nausea, a curious sensation of reawakening from death to life (Davidson, Marshall).

Subjective verbal reports such as those by Humphrey Davy, Peter Roget, Benjamin Blood, and William James were followed much later by human behavioral studies. Fred McKinney (1932) used N_2O in the study of central dissociation. In his review of the literature he discovered

that impressions of the effects of nitrous oxide, such as those by Davy, Roget, Blood, or James, were only qualitative statements, similar to those made by physicians on the effects of this drug. McKinney tested nine subjects, using 25% N_2O, on pitch discrimination, two-point threshold, visual acuity, auditory acuity, and weight judgments, as well as on learning nonsense syllables, word preference, opposites, and on free association.

> Under the gas, free association tended to become more peculiar, all reactions were slowed, learning ability decreased, suggestibility increased, tapping failed to show a decrement, and the senses did not show evidence of being more acute.
>
> (McKinney, 1932, p. 199)

Nitrous oxide reduced the tendency to use a common or trite association. McKinney ran three subjects under 50% N_2O, and found they could no longer read because everything looked blurry to them. Writing became larger and incoordinated. Other reactions were all slowed.

C. R. Marshall (1937), who had produced research on Indian hemp in 1897, mechanically recorded reaction times as well as aiming errors, and performed a number of experiments on remembering under nitrous oxide. He defined moderate intoxication, produced by 30% N_2O, as that dose which caused an increase of simple reaction time of 15% to 25%. He defined severe intoxication, produced by 45% N_2O, as that dose which increased simple reaction time 25% to 40%. Tasks to be used to assess

remembering included writing out the Lord's Prayer and answering 10 simple questions in writing.

As N_2O concentration increased, performance of the tasks took a longer time, and "troublesome points began to occur. Thus 'Lead us not into temptation' was momentarily left out of the Prayer" (p. 23). "With deeper intoxication the two chief stumbling blocks in the Prayer were at 'Lead us not into temptation' and 'Give us this day our daily bread,' and in this order." Above 50% N_2O

> the remembrance of the Prayer began to break down. The earliest part of the prayer was the most fixed in memory. The end portion, even the 'Amen,' was never remembered if memory for the middle part failed. When remembrance of all beyond 'Thy will be done' had gone the earlier part of the Prayer could still be repeated.

At 60% N_2O, memory of the first line began to fail. When a word series was listened to, as the inhalation of N_2O was beginning, "the earlier words, heard under slighter degrees of intoxication, were remembered better than those at the end of the series" (p. 26).

C. R. Marshall continued his research on nitrous oxide in a paper entitled "The Threshold of Unconsciousness" (1938). The dosage level of N_2O was at 70%, which produced unconsciousness in 4 minutes, 30 seconds. A period of excitement just preceded unconsciousness. "Disinclination for work may be said to characterize all grades of intoxication" (p. 424). At 50% N_2O "incoordination of movements becomes noteworthy and with it begins a loss of all sense

of self." Reaction times showed the familiar increase in length at low doses of N_2O. As N_2O increased, variations from the average increased reaction time became larger and more frequent. At the borderline of unconsciousness, "the responses tended to be violent. Considerable persuasion and even intimidation were then often required to obtain them" (p. 425). "Vision was apparently impaired at this stage" (p. 426).

At these high dosage (50% N_2O) levels, "a qualitative change of character occurred. . . . It had a character of omniscience and commenced with an exaggerated estimate of one's own abilities." The subject wrote "Intellectually I feel as keen as ever . . . I feel capable of conducting the Government . . . If I were Foreign Secretary I could set the international muddle aright" (1935).

> On the verge of the threshold [the subject] wrote "Time is beginning to have no meaning for me . . . The effect experienced [at 60% N_2O] was one almost of non-existence. . . . On recovering from the unconsciousness of 70% N_2O to the consciousness of 50% N_2O in a continuous experiment, I had the curious experience of an awakening from death to life. . . . Late in the research I became imbued with the thought, especially in the early morning after awakening from sleep, that the experiments I had been conducting were of great educational value." (p. 429)

After C. R. Marshall's death there was little research upon behavioral effects of nitrous oxide. Some investigations were undertaken on nitrogen narcosis, and on the effects of carbon dioxide and of cold combined with

pressure, by Case and Haldane (1941). A thorough review of Theories of General Anesthesia, by Thomas Butler, appeared in 1950. The next research on human behavior under nitrous oxide was done by Hannah Steinberg in 1954. She examined the effects of 30% N_2O upon cognitive behavior, expecting to find a deterioration under the drug condition, and expecting this deterioration to selectively impair more "complex" behavior to a greater degree than the simple motor tasks. As expected, performance on all tasks was impaired by 30% N_2O; however, the "Ball-bearing" task was the task most sensitive to the drug. This task, which was also used by Case and Haldane (1941) in their study of nitrogen narcosis, consists of inserting "with a pair of forceps steel balls as quickly as possible into a vertical tube" (Steinberg, 1954, p. 172). The dependent variable was the number of balls inserted in 40 seconds. The next most impaired tasks were two multiple choice tests, "Verbal Analogies" and "Non-Verbal Analogies." The next most impaired task was another motor task, "Dotting," which embodied "a revolving spiral of irregularly placed dots at which the subject aimed with a pencil." Two mental tasks followed, "Arithmetic" (written) and "Digit Span, Backwards" (verbal). The next most impaired task was a motor task, "Tapping," and finally three slightly impaired verbal tasks, "Fluency Flowers," "Fluency Things to Eat," and "Digit Span, Forwards."

Thus Steinberg found that N_2O did not impair skills in a strictly hierarchical manner, but instead showed impaired performance scattered fairly evenly among motor, written, and verbal skills.

Subjective impressions of time estimations did not correlate with performance tasks of time estimation (Steinberg, 1955). Subjects reported feeling far away, detached, with impressions of vivid imagery. Some felt flashes of insight.

Nitrous oxide produces analgesia, freedom from pain, and produces a tendency to forget about the presence of pain, and to attach little importance to the presence of painful stimuli (Parkhouse, 1959). Nitrous oxide produces pronounced subjective changes even when performance is undisturbed on such tasks as Peg-board, picture arrangement, and the Stroop colour-word test (Frankenhaeuser and Järpe, 1962).

Thirty percent N_2O caused subjects to slow down, and also to make more errors on identifying opposites (verbal), multiplication, inductive problems of 4-letter groups, and counting blocks in a drawing (Frankenhaeuser and Beckman, 1961). Nitrous oxide diminishes the response evoked by an auditory signal, as measured by an EEG; evoked response time is slowed and amplitude is lowered (Jarvis, 1971). Hand-tool dexterity and Peg-board performance was not impaired at 30% N_2O (Biersner, 1972). The Wechsler

Memory Scale showed no impairment of orientation, mental control, digit span or short term visual recall under 30% N_2O. Short term memory and associative learning were impaired. N_2O seemed to impair short term memory for simple geometric figures (Biersner, 1972). N_2O at 33% evokes analgesia, freedom from pain, while not altering a subject's willingness to label a stimulus as hot (C. R. Chapman, 1973). N_2O at 30% did not impair vigilance or sustained attention in 8 out of 12 subjects, who performed perfectly (Garfield, 1975). If a subject had formulated a decision strategy, then 30% N_2O did not impair performance on this task. No coding errors were made on the digit symbol substitution test. Reaction times were slowed (Garfield, 1975). Choice reaction time (Bradley, 1976) was slowed from 0.41 sec to 0.50 sec under 30% N_2O. Simple reaction time was slowed from 0.25 sec to 0.32 sec under 30% N_2O. There were profound subjective sensations (Bradley, 1976).

Having reviewed human behavioral studies of the effects of nitrous oxide, I shall next review physiological effects of nitrous oxide. After that I shall turn away from considering N_2O effects upon the living creature, and instead start to consider the nitrous oxide molecule itself. In so doing, I shall be comparing nitrous oxide to other analgesics, to euphorics, and to the anxiety-reducing drugs.

CHAPTER V

THE PHYSIOLOGICAL EFFECTS OF NITROUS OXIDE

Both Humphrey Davy in 1799 and C. R. Marshall in 1938 describe breathing nitrous oxide to unconsciousness. Marshall used a dosage level of 70% N_2O. It is essential to breathe oxygen with the nitrous oxide, since there is no available oxygen in the nitrous oxide molecule. Breathing 100% nitrous oxide has produced death in rats within 45 seconds.

Until 1956, nitrous oxide appeared to be completely safe to use for any length of time, as long as adequate oxygen was breathed with the N_2O. The first indication that nitrous oxide might alter the immune response appeared in an article by H. C. Lassen (1956). The patient, a fifteen year old boy with fulminant tetanus, was undergoing convulsions and rigidity, with the danger that he might suffocate from laryngeal spasms, or a complete tetanic standstill of the respiratory muscles. The previous conventional treatment included barbiturates, chloral hydrate and d-tubocurarine chloride. Lassen chose to also use 50% to 60% nitrous oxide, in order to lessen the need for curare and the sedatives, and to lessen the great emotional strain of being unable to communicate

because of the curare paralysis. Unfortunately, the boy developed aplastic anemia, with a Hemoglobin of 80% of normal. Leucocytes (white blood cells) fell from a normal level of about 14,000 down to 1,200 per cubic millimeter. Granulocytes fell to 10% of normal. Despite ten blood transfusions and five different antibiotics, he developed septicaemia and myocarditis, and died on the 29th day.

One more patient died before Lassen realized that nitrous oxide was the common factor in these sudden blood cell changes. Other patients either had not been given N_2O for so many days, or the N_2O was halted as soon as their blood cells showed this loss. Eastwood (1963) tested the effects of 80% N_2O for six days on albino rats. White blood cells fell from a normal count of 13,000 to 1,130. Polymorphonuclear cells disappeared first. Bone marrow showed a total cessation of reproduction of cells, with mitosis disappearing. Eastwood tried nitrous oxide as a last resort in cases of myelogenous leukemia, since these patients had white blood cell counts of 170,000 and 181,000, far too high. N_2O did bring the white blood cell count down, but the patients died from intracranial hemorrhage and a high fever.

PHYSIOLOGICAL EFFECTS OF N_2O

There are a number of physiological in vivo responses to nitrous oxide which are immediately measurable and are very puzzling to explain. Some researchers have proposed that nitrous oxide is an α-adrenergic agonist, that is, that N_2O potentiates or releases plasma norepinephrine, an excitatory substance that we subjectively experience as "a rush of adrenaline." Evidence is conflicting and the cardiovascular responses are extremely complex. Craythorne and Darby (1965) showed that nitrous oxide produced an immediate decrease in ventricular force, without causing a change in blood pressure, heart rate, or cardiac output, in dogs. Lundberg (1966) found that N_2O increased heart rate but reduced stroke volume in dogs.

N. Ty Smith (1966) found that nitrous oxide caused no cardiovascular changes except for a rise in total peripheral resistance in dogs. R. A. Millar (1969, 1970) found that N_2O produced an increase in sympathetic discharges in cats. J. H. Eisele (1969) found that nitrous oxide produced a reduction of the maximum aortic acceleration within 8 seconds of the first breath in dogs. Nitrous oxide reduced the heart rate and stroke volume, but their was also a slight rise in mean arterial blood pressure.

N. Ty Smith (1970) found that nitrous oxide caused

an increase in mean arterial pressure, in right atrial pressure, in systemic vascular resistance, and in forearm vascular resistance, in humans. Forearm blood flow was reduced. Pupils dilated. Plasma norepinephrine levels rose. Leighton (1973) concluded from Ty Smith's studies that there is good evidence that nitrous oxide is an α-adrenergic agonist.

PHYSIOLOGICAL EXPERIMENTAL STUDIES ON N_2O

Besides its effects upon the immune response, and its possible action as an α-adrenergic agonist, nitrous oxide affects the process of mitosis in profound and mysterious ways. Humphrey Davy (1799) was the first to notice that plants will not grow, seeds will not germinate, in an atmosphere containing nitrous oxide. The first modern indication that nitrous oxide might produce changes in the process of mitosis came when Ostergren (1944) tested this analgesic gas upon the pea plant Pisum sativum. N_2O halted mitosis in metaphase.

In 1950, Ferguson was able to induce polyploidy in the onion plant Allium cepa, but only at a pressure of 6 atmospheres. In 1958, Ebert and Hornsey speculated that inert gases, such as nitrous oxide, seem to compete with oxygen for access to as yet unidentified, but specific, sites within the cell, perhaps within the nucleus.

In 1963, Green and Eastwood noted that rats and

humans are similar in their response to nitrous oxide; both exhibit depression in their nervous systems and in their haemopoietic (blood-forming) systems. In 1964, Rector and Eastwood showed that N_2O (high levels) is lethal to the chick embryo. In 1965, Smith, Gaub, and Moya noted the teratogenic (causes deformities) effects of anesthetic agents, among them, N_2O. In 1966, Fink and Kenny found that N_2O decreases the rate of mammalian cell proliferation in monolayer culture. In 1967, Fink, Shepard and Blandau studied the teratogenic activity of N_2O. The effects that nitrous oxide produces on mitosis have ramifications far beyond tissue culture procedures or hybrid crop improvements. Embryos do not grow normally in pregnant rats who are exposed to 50% N_2O (Fink et al., 1967). Even 2 days in 50% N_2O is enough to cause death in 19% of the embryos who are then resorbed. Resorptions rise to 25% after 4 days of nitrous oxide, and rise to 57% after 6 days of nitrous oxide. Weight of embryo falls from 3.7 g to 2.7 g. Every embryo who survived had defective vertebrae. Some vertebrae had no center of ossification on the affected side. Some ribs were fused. Adjacent vertebrae were fused together between their ossification centers. Three embryos were hydrocephalic. One had a damaged kidney; one had an enlarged heart. Half as many males (35) as females (70) survived. More than half of the male embryos were absorbed. Nitrous oxide appears

to be selectively lethal for males. The rats in the study of Fink et al. (1967) were treated with 50% N_2O, 25% O_2, and 25% N_2 from day 8 to day 12 of the pregnancy, too early for gonadal differentiation. Fink et al. thought that N_2O affected skeletal growth through direct gene action.

Green (1968) investigated the effect of N_2O on RNA and DNA content of rat bone marrow and thymus. Bone marrow is involved in the creation of red blood cells and white blood cells. The thymus gland is involved in changing immature white blood cells into immunologically competent white blood cells, those that can recognize and bind a foreign substance, an antigen.

Snegireff, Cox, and Eastwood (1968) investigated the effect of N_2O on the developing chick's embryo, noting weight, mortality, and gross anomaly rate, as well as the neural tube mitotic index. Mitotic rate is a ratio of the number of cells that enter mitosis, to the total number of cells. A normal mitotic index is 0.03, as about 3% of the total cells will be entering mitosis at any one time. Low temperatures cause growth rate to approach zero; the mitotic index in this condition increases to 0.36. Thirty-six percent of cells have entered mitosis but are unable to complete cell division. Colcemide causes the mitotic index to climb to 0.65. A pH of 7.7 (slightly basic) at 29 degrees Celsius brings the mitotic index

up to 0.44. Cells enter metaphase and then remain there. N_2O at 5.1 atmospheres (Rao, 1968) upon mammalian carcinoma Hela cells caused an increase in mitotic index from 0.03 up to 0.62 after 16 hours. Growth was blocked in metaphase. Nitrogen, N_2, at similar pressure caused no metaphase block. If cells are treated with thymidine, a normal constituent of DNA, they will all start to enter mitosis together, after 3-3/4 hours. If, at that point, the cells are placed in N_2O at 5.1 atmospheres, the mitotic index will rise from zero to 0.94 in 8½ hours. This N_2O block is reversible; when N_2O is removed, cells can now complete mitosis. About 90% of cells completed mitosis one hour after N_2O was removed (Rao, 1968).

Johnson (1971) kept rats in an atmosphere of 80% N_2O for six days, noted the characteristic changes in blood cell counts, and then kept them in room air for days 7-11. Reticulocyte count, which had fallen to 30% of normal, recovered, and in fact rose to 168% of control levels. Phagocytes recovered their normal mobility. Red blood cell production, as measured by incorporation of radioactive iron, recovered from a low point of 25% of normal to a "rebound" high level of 225% of normal. Cell division, mitosis, as measured by incorporation of radioactive ^{14}C-thymidine into DNA in the chromosomes of white blood cells, recovered from a low point of 25% of normal to a high level of 125%. Johnson hypothesized that these

changes were due to the ability of nitrous oxide to function as a free radical scavenger, but he could find no causal relationships between the tissue content of free radicals and the blood cell changes brought on by nitrous oxide.

Brinkley and Rao (1973) again used Hela cells whose division had been synchronized with thymidine, and placed these in N_2O under a pressure of 80 lb/in^2. If the N_2O treatment lasted for 4 hours, cells recovered afterward and proceeded through mitosis. However, if N_2O treatment lasted for 12 or 16 hours, cells did not recover. Bipolar cells, those dividing normally into two daughter cells, usually comprise 96% of the population, with a few tripolar or tetrapolar. After 12 or 16 hours under N_2O, incidence of bipolar cells had fallen to 55%, while tripolar cells rose to 27%, and tetrapolar cells rose to 13%. After 36 hours of N_2O, the count of bipolar cells had fallen even lower, to 40%, while tripolar cells rose to 42%, and tetrapolar cells rose to 17%. Cells with two nuclei, an abnormal condition which follows the tetrapolar state, usually comprise 3.75%; after N_2O, binucleate cells comprise 13.5%.

In 1973, Corbett et al. studied the effects of low concentrations of N_2O in rat pregnancy. Even 100 ppm of N_2O for 8 hours a day produced a high fetal death rate of 14.5% to 18.4% when the days of exposure were the 4 days from day 10 to day 13, or the 6 days from day 14

to day 19 of the rat pregnancy. In 1974, Bussard et al. showed fetal changes in hamsters exposed to N_2O.

PHYSIOLOGICAL EFFECTS ON HUMANS

In 1960, Triconi, Serr, and Solish observed that nitrous oxide had an adverse effect upon human embryos, causing selective mortality of males during early pregnancy. By 1968, it was recognized that anesthetics like nitrous oxide might create a special hazard for those who work with them, and Bruce, Eide, Linde, and Eckenhoff (1968) studied the causes of death among anesthesiologists--a 20 year survey. Lymphoid cancers were one of the leading causes of death.

In 1969, Linde and Bruce measured ppm of anesthetic gases in operating rooms, indicating the occupational exposure of anesthetists to N_2O and other anesthetics. David Bruce wrote a review article on changes in the immune response brought on by anesthetics, including N_2O, but again the mechanism of action is not understood (Bruce, 1971). Bone marrow fails to produce the normal numbers of white blood cells and red blood cells. He commented on the seriousness of this problem.

In 1971, Cohen, Belvill, and Brown published an article entitled "Anesthesia, Pregnancy, and Miscarriage--A Study of Operating Room Nurses and Anesthetists." Exposed operating nurses had 29.7% miscarriages compared

to control nurses' rate of 8.8%. Exposed female anesthetists had 37.8% miscarriages compared to control female doctors' rate of 10.3%. In 1972, Pfaffli, Nikki, and Ahlman showed that anesthetics, including N_2O, not only exist in operating room air, but also in "end-tidal air and in venous blood of surgical personnel." They showed that there is chronic exposure to such anesthetic gases in the operating theater and in the recovery room.

In 1972, Knill-Jones and Moir, and Rodrigues and Spence made a control survey of woman anesthetists in the United Kingdom, correlating practice with problems of pregnancy. Exposed women anesthetists had 18.2% spontaneous abortions compared to control female physicians who had 14.7% spontaneous abortions. The women anesthetists had 5.5% congenital abnormalities of infants compared to 3.6% for the infants of female physicians.

In 1974, Corbett, Cornell, Endres, and Leading documented birth defects among children of nurse anesthetists. In 1974, Cohen et al. made a national study of occupational disease among operating room personnel. In 1974, Bruce et al. showed that even trace anesthetics including N_2O at 50 ppm, produce decrements in perceptual, cognitive, and motor skills.

In 1974, Millard and Corbett measured N_2O concentration in the dental operatory. In 1975, Knill-Jones et al. made a control survey of male anesthetists in the

United Kingdom, correlating anesthetic practice of the husband with problems in their wives' pregnancies. In 1975, Cohen et al. made a survey of anesthetic health hazards in pregnancies of dentists' wives. Wives of exposed dentists had a spontaneous abortion rate of 16% compared to a rate of 9% for wives of unexposed dentists. In 1975, Lecky measured anesthetic trace levels in 98 U.S. hospitals. In 1976, Bruce and Bach showed the trace effects of anesthetic gases on behavioral performance of operating room personnel. In 1976, Kripke et al. studied in rats testicular reaction to prolonged exposure to 20% N_2O. After 14 days they could see damage to seminiferous tubules, and multi-nucleated spermatozoa.

There are about 25,000 hospital operating rooms and each year about 20 million patients are anesthetized. Nitrous oxide is the most widely used inhalation anesthetic gas. Every day about 50,000 hospital operating room personnel breathe traces of N_2O and other gases. In dental offices (100,000 dentists and assistants) about 4.5 million patients are anesthetized with N_2O and supplementary drugs every year. The usual occupational exposure level for N_2O is 400 ppm to 3,000 ppm. Dental offices have levels of 5,900 ppm to 6,800 ppm (Occupational Exposure to Waste Anesthetic Gases and Vapors, DHEW, NIOSH, March 1977).

To summarize, nitrous oxide affects the immune response, it seems to be an α-adrenergic agonist, and

it causes abnormal mitosis, fetal death, and congenital abnormalities in experimental animals and in human beings.

In order to continue exploring further the effects of nitrous oxide, we must shift our focus from in vivo research on humans or animals to in vitro research upon cells, and particularly upon neurons.

OXYGEN USE AND N_2O

At the time that Humphrey Davy discovered nitrous oxide in 1799, scientists were just beginning to understand that humans, animals, and plants need and use oxygen from the air, in order to stay alive. If a drug such as nitrous oxide at 70% can produce unconsciousness, does it do so by interfering with the transport or use of oxygen?

Nitrous oxide is absorbed by the surfacants on the inside of the lungs at the alveolar membrane (Stanaszek, 1972). As it is breathed into the lungs, some patients take note of the slightly sweet taste of nitrous oxide. Humphrey Davy also spoke of "a feeling in my lungs akin to taste." Nitrous oxide causes people to breathe faster. When resting, an average respiratory frequency is about 11 breaths per minute. With 30% N_2O, this rate rises to about 14 breaths/minute (Bradley, 1976). This increase from N_2O also happens with moderate exercise, from about 26 breaths/min to 30/min, and with strenuous exercise with N_2O from about 34/min to 39/min. Breathing is

slightly shallower; tidal volume increases with increasing levels of N_2O (Bradley, 1976). Liters per minute increased under N_2O, since even with shallow breaths, enough breaths per minute were breathed to cause an increase in respiratory minute volume, from 6.79 L/min to 7.02 L/min at 30% N_2O at rest. This increase was most pronounced with strenuous work, changing from 55.39 L/min to 59.45 L/min (Bradley, 1976).

Nitrous oxide enters the alveolar epithelial cells, crosses the subepithelial tissue, and enters the pulmonary capillaries. N_2O is 30 times more soluble in body fluids than is nitrogen, N_2. Because of this big difference in solubilities, N_2O pours into the body at a tremendous rate, while the N_2 leaves much more slowly. Humans absorb a liter (1,000 cc) of N_2O each minute until equilibrium is reached after half an hour of breathing a mixture of nitrous oxide and oxygen. Oxygen is used at a rate of 240 cc/min when resting. A typical N_2O/O_2 mixture is 2 liters/min of N_2O with 5 liters/min of oxygen.

When humans or animals are resting, they use less oxygen while breathing nitrous oxide, than they use while breathing air (Schatte, 1973, 1974; Bradley, 1976). This is not surprising since narcosis seems to slow down many processes, including metabolism. But <u>even</u> while exercising, both Schatte et al. (1974), and Bradley and Dickson (1976), found that humans used <u>less</u> oxygen while breathing nitrous

oxide than while breathing air. In these experiments nitrous oxide does seem to be interfering in some mysterious way with the transport or use of oxygen.

Nitrous oxide is often called the "first gas" in articles by anesthesiologists, and the actual anesthetic, such as halothane, is called the "second gas." Because N_2O rushes in so fast, it drags the "second gas" into body tissues faster than that gas would enter ordinarily. N_2O produces this "second gas effect" with halothane (Epstein, 1964), with ethylene and with cyclopropane (Stoelting, 1969) and with carbon dioxide (Kitahata, 1971). Carbon dioxide is not, of course, given as an anesthetic; but it is the gas we breathe out, and when we breathe in N_2O, the N_2O enters so fast that it keeps the carbon dioxide in our alveolar capillaries. Whitteridge and Bulbring (1944) noted that N_2O caused an increase in the discharge frequencies of pulmonary inflation receptors and in pulmonary deflation receptors. Perhaps this is due to the CO_2 second gas effect. Perhaps N_2O somehow sensitizes these receptors.

Nitrous oxide rushes into alveolar tissue, into capillaries and the bloodstream, and from there to every part of our bodies, including nervous tissue. Subjects report "I feel different now," with the second or third breath of 25% to 30% N_2O. Such subjective effects are felt even sooner with higher concentrations of nitrous oxide.

In the bloodstream, N_2O is absorbed by serum albumin, a blood protein (Stanaszek, 1972). Nitrous oxide does not appear to bind to hemoglobin, nor to affect the ability of hemoglobin to carry oxygen (C. Waltemath, 1971). N_2O does not cause any change in the spectrophotometric curve of hemoglobin with oxygen. There is no change in the Bohr effect, an interaction between hemoglobin and the dissolved carbon dioxide within the red cell. There is a transient effect of N_2O on red blood cells in vitro. First they shrink from the initial N_2O. Then they swell again (Longmuir and Grace, 1970). Erythrocytes (red blood cells) do not seem to be impaired in their ability to retain potassium ions (K^+) in spite of this shrinking and swelling. Platelet aggregation is inhibited 34% by 64% N_2O (Ueda, 1971).

The serum enzymes such as serum transaminase, dehydrogenase, aminopetidase, phosphatase (Boehmer, 1970), serum proline hydroxylase, alkaline phosphatase and alanine amino transferase (Stein et al., 1972), appear to be unaffected by N_2O. Tyrosinase is inhibited by N_2O (Behnke et al., 1969). Aspartate amino transferase levels rise (Stein et al., 1972).

There is a temporary rise in blood sugar level (Atkins and Thornburn, 1971), and an increase in free fatty acids (Allison et al., 1969). Insulin levels fall (Allison, 1969). There is some controversy over whether

nitrous oxide is an α-adrenergic agonist, that is, whether it causes a sudden release of adrenaline (epinephrine) from our adrenal glands into our bloodstream, thus preparing us for "fight or flight." Norepinephrine (N.E.) is a neural transmitter which may also be released by nitrous oxide. This has an excitatory effect upon a wide range of neurons in the brain as well as in the heart (Goodman and Gilman, 1975). Experiments concerning the effect of nitrous oxide upon postganglionic adrenergic nerve endings have been performed using the stellate ganglion and related heart-regulating nerve centers. These results were summarized earlier in this chapter under the heading, "Physiological Effects of N_2O."

A number of different researchers have investigated the effect of nitrous oxide upon the firing rate of different groups of neurons. Mori et al. (1972) found that N_2O produced no consistent change in neuron firing of thalamic relay nuclei. Kitahata et al. (1973) found that N_2O reduced the firing of central trigeminal nociceptors, but N_2O increased the firing rate of nucleus caudalis neurons. Sasa et al. (1967) found that N_2O reduced the firing rate in the inferior colliculus and in cortical evoked click potentials by 20%.

Some researchers have investigated the interaction of nitrous oxide with the cortical response to monoamines, to norepinephrine, to 5-hydroxytryptamine, and to

acetylcholine (E. Johnson et al., 1969). Others have used such a microelectrophoretic technique with gamma amino butyric acid (GABA) and with assorted amino acids as well as acetylcholine on the cortical cerveau isolé (Crawford, 1970).

Hills (1972) found that spontaneous neuron firing was increased by nitrous oxide, which caused cellular dilation by disturbing the osmotic regulation. Gottlieb et al. (1968) found that N_2O interfered with sodium active transport in sciatic nerve and across frog skin.

In trying to understand the complex effects of nitrous oxide, I have so far considered its effects on lung tissue, on α-adrenergic release on receptors, on heart function, on some brain neuron groups, and on the interaction of nitrous oxide with transmitter substances and brain metabolites. As we consider the effect of N_2O upon neurons we are moving from a focus upon the whole organ, group of cells, or individual cells, inward to concentrate upon the living cell itself.

CHAPTER VI

NITROUS OXIDE ANALGESIA AND OTHER ANALGESICS

Berkowitz et al. (1976) wished "to characterize the nature of nitrous oxide analgesia." As a dependent variable for pain, he used the phenylquinone writhing test: a peritoneal injection of phenylquinone, and then a count of the number of writhes exhibited by the mice during a five minute period.

40% N_2O lowered the number of writhes by 26%.
60% " " " " " " " 45%.
70% " " " " " " " 62%.
80% " " " " " " " 84%

Naloxone and naltrexone, both morphine antagonists, also antagonized the analgesia produced by nitrous oxide. Naloxone or naltrexone alone had no significant effect upon writhing. But naloxone or naltrexone, given just before the nitrous oxide, reduced the analgesia. Instead of lowering the number of writhes,

60% N_2O with naloxone produced no change in writhes
60% " " naltrexone reduced writhes only 12%
70% " " naloxone " " " 10%
70% " " naltrexone " " " 21%
80% " " naloxone " " " 24%

80% N_2O with naltrexone was not measured. Naloxone or naltrexone dosage level had to be at 5 mg/kg to be effective.

N_2O analgesia was not only antagonized by naloxone or naltrexone, it was also made ineffective by rendering the mice tolerant to morphine. Morphine was given in an increasing dosage:

30 mg/kg twice on day 1

50 mg/kg twice on day 2

60 mg/kg three times on day 3.

On day 4, no morphine was given; phenylquinone was given, and the number of writhes were observed with and without N_2O. An additional control group of mice had been given saline injections in a regimen paralleling the morphine injections. The previously given morphine did not have any lingering analgesic effect. Morphine tolerant mice writhed 60 and 56 times. Saline injected mice writhed 53 and 57 times. In the saline-treated mice, 80% N_2O reduced writhes by 93%. In the morphine-tolerant mice, 80% N_2O reduced writhes by only 16%. Berkowitz et al. (1976) concluded from these experiments that N_2O may release or potentiate one of our endogenous opiates, enkephalin or β-endorphin. The other explanation offered is that naloxone or naltrexone may antagonize any analgesic, not only the opiate alkaloids.

Relief of pain is complicated to investigate

experimentally. It is difficult to synthesize the various drug effects with the analgesic effects of hypnosis, acupuncture, and electrical stimulation of periventricular brain areas. Although Berkowitz et al. chose the phenylquinone writhing test, other investigators have used a hot-plate tail-flick test. In this test, a response to pain occurs in a few seconds; if analgesia prevents the tail-flick response, the stimulus is automatically turned off before the animal is physically hurt. This test is more sensitive to momentary pain or analgesia, and is not confounded by contractions occurring in intestinal smooth muscle. Opiates act directly on smooth muscle cells to inhibit contractions, as well as relieving pain from any source. Phenylquinone causes contractions as well as pain. Nitrous oxide relieves pain but has no special effects upon smooth muscle.

Since the discovery of enkephalin and β-endorphin, investigators have leaped upon the bandwagon, attempting to explain all analgesia by a binding of the drug to the endogenous opiate receptor protein, or by a release of the endogenous opiate. Nitrous oxide does not seem to bind to any protein. It "potentiates" a large number of drugs, including all the anesthetics. This word "potentiates" has simply been defined as: the same behavioral effect is produced, using N_2O plus a <u>lower</u> dosage level of the stronger drug, as is obtained using

a higher dosage level of the strong drug alone.

To test whether N_2O releases enkephalin or β-endorphin one would have to perform a much more sensitive experiment, perhaps implanting micropipettes into periventricular areas of the animals' brains. One would have to ascertain normal baseline values for enkephalin and β-endorphin, which would undoubtedly show a wide variation among the individual animals. Then N_2O could be given at different levels, and any changes noted in the amounts of enkephalin and β-endorphin released. N_2O dosage levels needed for sufficient analgesia vary widely among humans (23% to 60%), a further complicating factor.

Akil et al. (1976) electrically stimulated periventricular neurons in rat brains, and believed that such stimulation produced analgesia by increasing the release of endorphin from these neurons. The critical experiment by Akil, Mayer, and Liebeskind (1976) supporting this hypothesis, offered as evidence the blocking of analgesia from electrical stimulation by naloxone. Mayer and Hayes (1975) produced acupuncture analgesia in humans, rotating needles at the base of the thumb to block pain from electrical stimulation of a tooth-pulp cavity. This effect was blocked by naloxone. Stress itself produces analgesia that is not abolished by naloxone (Mayer and Hayes, 1975).

Hayes (1977) offers some cautionary thoughts on all these experiments. Naloxone antagonism is the primary

evidence cited by those investigators who seek to unite all analgesic effects under the common mechanism of the release of endogenous opiates. Naloxone blocks the analgesic effects of electrical brain stimulation, acupuncture, N_2O, lanthanum, marijuana, and acetylcholine, as well as blocking other responses produced by cholinergic agents, glutamate and dextroamphetamine (Hayes et al., 1977). Naloxone thus may activate some opposing system rather than merely competing with endogenous opiates for the opiate receptor protein sites.

Naloxone antagonism may be a necessary condition to show that endogenous opiates are released. But naloxone antagonism is not a sufficient cause to infer that analgesia is produced only by an endogenous opiate release and binding. Hayes feels this cautious "approach should be given more explicit attention in current behavioral and physiological research" (Hayes et al., 1977).

Even while we are considering the analgesic effects of nitrous oxide, we must also keep in mind its remarkable effects in producing euphoria and amnesia, in reducing anxiety, and in halting cell division in metaphase. Even if N_2O produces its analgesia by releasing endogenous opiates, we must search more deeply into the effects N_2O produces upon neurons if we hope to explain its other qualities. The list of drugs that produce effects similar to those of nitrous oxide is a formidable one, and includes

molecules which are chemically different and which produce different behavioral and experiential effects.

Analgesia for pain of low intensity is accomplished by aspirin (acetylsalicylic acid) and by tylenol (acetaminophen). Aspirin works on the peripheral nervous system, at the site of origin, as well as on the central nervous system. Aspirin inhibits the synthesis of prostaglandins that occurs in inflamed tissues. This inhibition prevents mechanical or chemical stimulations from sensitizing the pain receptors (Woodbury and Fingl, 1975). In the central nervous system, investigators have suggested that aspirin acts upon the hypothalamus, lowering fever as well as pain. Aspirin does not appear to affect the reticular activating system. Aspirin is about 1/10 as strong as codeine, as an analgesic. Aspirin affects the electron transport chain of proteins within the mitochondria, uncoupling the synthesis of ATP from the transport of electrons (Miyahara, 1965). The result of this is an increased need for oxygen and an increased production of carbon dioxide. Aspirin also causes the release of epinephrine from the adrenal medulla. Tylenol, like aspirin, acts upon the hypothalamus in reducing fever.

Xylocaine is the analgesic commonly injected locally at the dentist's office. Such local anesthetics block nerve conduction in every type of nerve fiber. Several different mechanisms of action have been suggested.

Perhaps xylocaine interferes with the binding of Ca^{++} ions. The site of action may be at the inside of the cell membrane. Maybe xylocaine increases the surface pressure of the lipid layer, thus closing the "pores" of the neuron cell membrane (Shanes, 1963). Maybe xylocaine increases the degree of disorder in the neuron lipid cell membrane (Metcalfe, 1968).

Cocaine resembles xylocaine and the other local anesthetics in all these effects. In addition, cocaine blocks the uptake of catecholamines at adrenergic nerve endings. Catecholamines are the "excitatory" neuron transmitters that cross the synapse from one neuron to the next. Catecholamines include ephinephrine (adrenaline), norepinephrine, and dopamine. Cocaine, as well as other local anesthetics, also inhibits cell division in sea urchin eggs.

Cocaine, since it blocks re-uptake of norepinephrine and the other catecholamines, produces excitement like the amphetamines. The user experiences this subjectively as a happier mood, a sense of alertness and increased energy, restlessness, and garrulousness. Cocaine addicts describe this euphoria in the same words as are used by amphetamine addicts, buyers and lovers of "speed." The users feel their mental powers are increased. "The user feels fascinated or preoccupied with his own thinking processes and with philosophical concerns about 'meanings'

and 'essences'" (Jaffe, 1975). Animal amphetamine users show stereotyped behavior which is thought to involve dopaminergic structures in the corpus striatum. Animals will self-administer cocaine in a cyclic pattern of use and abstinence.

Later, in Chapter VIII, I will describe the enkephalins and endorphins and their "opiate" receptor proteins which exist in neurons of the periventricular areas of the brain. Current hypotheses about the opiate alkaloids, morphine, heroin, percodan, and codeine, suppose that these drugs are bound by the opiate receptor proteins and this results in analgesia and euphoria.

EUPHORIA AS AN ANALGESIC RESPONSE

Commentators and reviewers often speak scornfully of the sense of wonder expressed by those writers who try to describe the effect of a drug such as opium or nitrous oxide. In their haste to condemn the use of drugs which have led to physical addiction, many reviewers have also belittled the experience of euphoria.

To belittle the experience of euphoria shows a lack of understanding of the importance of euphoria as a part of the complete effect of analgesia. Subjective aspects of the experience of pain, when it is possible to separate this factor from the physical stimulus, play a crucially important role. The most powerful analgesics do more

than simply dull the painful sensation or eliminate the direct sensation. They act by affecting both the physical components and the psychological components of the experience of pain. Nitrous oxide has this double aspect, both lowering physical pain locally and lowering general anxiety. The sensation of physical hurt seems attenuated, dulled, or eliminated. The feelings of psychological pain, which we name "anxiety" or "fear," are also attenuated. This change is reported in paradoxical statements by the patient, such as "I feel the pain but I don't care (laughs)." Drugs which produce this kind of euphoria with analgesia induce feelings of well-being, excitement, or pleasure.

When we consider the problem of addiction, these two components of pain are seen most clearly. Obviously a person who suffers physical pain may become an addict of a powerful analgesic. We even sympathize with such a problem. However, people who suffer from chronic free-floating anxiety and fear may also become addicted to such drugs. Psychological pain drives these people to crave the drugs that will bring them euphoria. Our cultural attitude, our "Puritan ethic," condemns such sufferers, as well as prevents us from understanding euphoria as an analgesic response.

Those writers who are describing euphoria go beyond the dictionary definition, "a sense of well-being and bouyancy" (Webster's, 1977, p. 344). There seem to be

a number of different subjective effects included in the experience of euphoria. Perhaps a lowering of anxiety is the most central feeling. This dimension is described as a sense of peacefulness; one is past caring; writers here go on to use the word sublime, a sense of bliss. A different dimension of euphoria is the sense of excitement, of intensity of one's emotions. Humans are willing to accept a degree of fear in order to experience excitement. The combination of fear and bliss in close temporal juxtaposition is such a novel sensation that humans seek out plants and chemicals that produce such effects. Even hallucinations may be sought out for their novelty, out of curiosity. Nitrous oxide seldom produces visual hallucinations; it often produces hallucinations of floating or sinking. Subjective sensations under N_2O are described by Roget (1799), Davy (1799), Blood (1874), and James (1882) (see Appendix).

The most complete description of the euphoria of opium has been given by Thomas de Quincey (1821):

> Dread agent of unimaginable pleasure and pain . . . the abyss of divine enjoyment thus suddenly revealed . . . a panacea--a soothing drug for all human woes . . . the secret of happiness . . . at once discovered . . . happiness might now be bought for a penny . . . portable ecstasies might be had corked up in a pint bottle; and peace of mind could be sent down in gallons . . . Nobody will laugh long who deals much in opium; its pleasures even are of a grave and solemn complexion; and in his happiest state, the opium eater even then speaks and thinks as becomes Il Penseroso.
>
> No quantity of opium ever did, or could,

> intoxicate . . . crude opium is incapable of producing any state of body at all resembling that which is produced by alcohol.
>
> The pleasure given by opium, when once generated is stationary for 8 or 10 hours; a case of chronic pleasure, a steady and equable glow. Opium introduces among the mental faculties the most exquisite order, legislation, and harmony. Opium greatly invigorates a man's self-possession. Opium communicates serenity and equipoise to all the faculties, active or passive.
>
> Opium gives, to the temper and moral feelings in general, simply that sort of vital warmth which is approved by the judgment.
>
> Opium gives an expansion to the heart and the benevolent affections. This expansion of the benigner feelings, incident to opium, is no febrile access, but a healthy restoration to that state which the mind would naturally recover upon the removal of any deep-seated irritation of pain that had disturbed and quarreled with the impulses of a heart originally just and good.
>
> Opium always seems to compose what had been agitated, and to concentrate what had been distracted (p. 73).

Opium has been used at least since 4000 B.C., and contains more than twenty alkaloids, which constitute 25% of the weight of powdered opium. Morphine constitutes 10% of the weight, codeine about 0.5% of the weight. Heroin is manufactured from morphine, about 2½ times as potent as morphine, and is rapidly converted into morphine in the body.

Methadone does not appear to resemble the opiate alkaloids when drawn as the two-dimensional chemical structure, but when folded into its three-dimensional form, it resembles morphine, and is bound to protein as is morphine. It is as strong an analgesic as morphine, it causes physical dependence, and its effect is blocked

by naloxone and naltrexone.

Demerol (meperidine) is a synthetic analgesic, chemically quite dissimilar to morphine, but also classed as a narcotic. In its three-dimensional form it also can fit into the opiate receptor protein. It produces euphoria as well as analgesia, and its effects can be antagonized by naloxone and other narcotic antagonists.

Fentanyl is a synthetic opioid which is 80 times as potent as morphine. Its analgesic and euphoric effects can be antagonized by naloxone and other opioid antagonists. Fentanyl is used exclusively for anesthesia. Sometimes fentanyl is given as preanesthetic medication, instead of morphine or demerol. Sometimes fentanyl is combined with a neuroleptic drug, droperidol; together these two drugs produce neurolept analgesia, a general quiescence, a state of seeming indifference to environmental stimuli. Given intravenously, and combined with nitrous oxide, they are potent enough for surgical operations. They do not produce sleep, nor unconsciousness, as usually defined for anesthetics. Induction is fairly slow, perhaps 5 minutes, and cannot be hurried by raising the N_2O level, because an induction delirium will occur. If there is postoperative respiratory depression, this can be counteracted by naloxone or other narcotic antagonists.

I have reviewed the behavioral effects and the hypothesized mode of action of all these analgesics: aspirin,

tylenol, xylocaine, cocaine, the opiate alkaloids, methadone, demerol, and fentanyl, in an attempt better to comprehend the analgesic action of nitrous oxide.

Nitrous oxide also produces a lowering of anxiety, calmness, sedation, a tranquil state of mind. Since it is a gas, bottled under pressure, needing valves, dials, and a mask to administer, N_2O is not prescribed for anxiety or nervousness as are the tranquilizers. Nevertheless, it is instructive to consider the mode of action of tranquilizers, in attempting thoroughly to study the mode of action of nitrous oxide.

ANTI-ANXIETY DRUGS

Tranquilizers, or anti-anxiety drugs, are prescribed more frequently than any other group of therapeutic agents. For reasons given earlier in this chapter when discussing euphoria, an anti-anxiety effect can be seen as a kind of analgesic effect, but dealing with the dimension of "psychological pain." "No consistent mode of action has been hypothesized for these drugs" (Goodman and Gilman, 1975, p. 188). Meprobamate (Miltown, Equanil) is the prototype, and one of the most widely used. It resembles phenobarbital in its effects. Its mode of action is unknown. It does not depress the reticular activating system. High doses (1600 mg) depress learning, motor coordination, and reaction time (Kornetsky, 1958; McNair,

1973).

McNair (1973) reviewed all studies of the effects of anti-anxiety drugs on human performance, and decided that no real conclusion could be drawn. Meprobamate suppresses REM sleep, as does phenobarbital and all barbiturates. Meprobamate cessation produces REM rebound, a temporary increase in the proportion of time spent in REM-state sleep. Tolerance and physical dependence develop.

Valium (diazepam) and Librium (chlordiazepoxide) are also used as anti-anxiety drugs, and work by some mechanism of action that is unknown. Of course, we cannot as yet define anxiety in any neurophysiological or biochemical way either. Valium and Librium do not suppress REM sleep. Tolerance and physical dependence develop. Paradoxically, there is an increase in hostility (DiMascio, 1973).

Quaalude (methaqualone) is used as an anti-anxiety drug even though it is classified as a hypnotic and sedative. The mechanism of action is unknown. Investigators disagree about whether Quaalude disturbs REM sleep. "In rat brain and mitochondria, it appears to compete with Krebs cycle intermediates for NAD-dependent enzymes" (Goodman and Gilman, 1975, p. 130). Tolerance occurs. Hangovers occur when quaalude is used as a hypnotic. Sweating is more profuse. Amnesia occasionally occurs.

Quaalude was rapidly taken up by drug abusers, who ascribed to it aphrodisiac qualities, and an increase in "open" communication. Some Quaalude addicts think it feels like heroin.

Dalmane (flurazepam hydrochloride) is another tranquilizer which is classified as a hypnotic and sedative. Low doses (30 mg) do not suppress REM sleep; higher doses (60 mg) may do so. No REM rebound occurs. Its mechanism of action is unknown.

Tofranil (imipramine hydrochloride) is a tricyclic antidepressant, so named because of its chemical structure. This is used in the treatment of depression, which is often mixed with anxiety in the patient. Tricyclic antidepressants do not actually cheer up a normal subject or elevate the subject's mood. The normal subject, given Tofranil, may complain about the difficulty of thinking or concentrating. The depressed patient, given Tofranil, may not be cheered up at once either. But after 3 weeks, they may feel more cheerful. Investigators have described the effects of Tofranil as a "dulling of depressive ideation" (Goodman and Gilman, 1975, p. 175). If used as a hypnotic, Tofranil causes hangover. Tofranil suppresses sleep. "All tricyclic antidepressants block the re-uptake of norepinephrine by adrenergic nerve terminals" (Goodman and Gilman, 1975, p. 176). Tolerance develops. Sweating is more profuse.

Thorazine (chlorpromazine hydrochloride) is not really used as an anti-anxiety drug, but rather is used in the behavioral control of diagnosed schizophrenic patients. Delay and Deniker (1952) felt that thorazine not only improved outward behavior but also moved brain processes away from psychosis and toward normality.

I shall in Chapter VIII review theories of anesthesia, since nitrous oxide has often been included among the gaseous anesthetics investigated by various theorists (Nahrwold, 1973; Pauling, 1961; Featherstone, 1963; Brauer, 1970; Roth, 1972; Johnson, 1973). I have reviewed behavioral studies of nitrous oxide analgesia, since N_2O is an excellent analgesic (Berkowitz et al., 1976; Parkhouse, 1959; Chapman, 1973). I have reviewed analgesia brought about by opiate alkaloids, by brain electrical simulation, by aspirin, xylocaine, cocaine, and other analgesics, noting when nitrous oxide produced an effect similar to these other drugs. I have also noted a mechanism of action proposed for a drug, which has also been proposed for nitrous oxide, for instance that it increases the degree of disorder in the lipid cell membrane: a theory for xylocaine (Metcalfe, 1968), and also for N_2O (Clements and Wilson, 1962; Roth, 1972; M. Johnson, 1973). I shall now turn to some of the other subjective, behavioral, and physiological effects of nitrous oxide. Nitrous oxide produces amnesia, a temporary forgetting of which road to take home.

N_2O produces a psychedelic effect, a change in perception, such as a sensation of "floating." Nitrous oxide is a spindle poison, that is, it halts mitosis in metaphase. In the next chapter, I shall review other drugs which produce amnesia or psychedelia, or act as spindle poisons.

CHAPTER VII

OTHER PROPERTIES OF NITROUS OXIDE: AMNESTIC PSYCHEDELIC, AND SPINDLE POISON

Besides its function as an anesthetic and an analgesic, nitrous oxide also occasionally produces a temporary amnesia. Scopolamine, one of the belladonna alkaloids, regularly produces amnesia. Scopolamine acts by competing with acetylcholine, the normal nerve-muscle transmitter molecule, which is also used in the sodium-potassium active transport system of neuron membrane. Morphine and scopolamine, a mixture called "twilight sleep," used to be given for childbirth, in order to produce both analgesia and amnesia.

Nitrous oxide produces a psychedelic state (see Chapter III, p. 31). Some psychedelic effect is produced by a wide variety of drugs, such as alcohol, ether, morphine and other opiate alkaloids, cocaine, psilocybin, marijuana, mescaline, and LSD. These drugs are chemically of such different structures that it seems unlikely that they would share any common mechanism of action. Alcohol blocks peripheral nerve conduction, but only at a high concentration (Israel, 1971). It is thought to act upon the reticular activating system (Himwich, 1972). The

mechanism of ether is as yet unknown. I have reviewed the mechanism of action of morphine and the other opiate alkaloids, and the subjective state of opium intoxication. I have reviewed possible mechanisms of action for cocaine, and its subjective effects. Psilocybin occurs in wild mushrooms of the genus Psilocybe. Mescaline is from the cactus plant Lophophora williamsii (peyote). These drugs may produce their subjective effects, when the receptor protein for the normal transmitter, 5-hydroxy-tryptamine, instead binds psilocin, the metabolite; or the receptor protein for norepinephrine instead binds mescaline. The normal brain transmitters are more water-soluble, less lipid soluble, than these two "transmitter mimikers."

LSD, lysergic acid diethylamide, produces a wide variety of unusual subjective effects. "Several feelings may seem to coexist at the same time" (Jaffe, 1975, p. 310). We are not usually aware of the fact that, in order to see the new, current pattern or image, our eyes and brain, having registered the past image, must "erase" it, toss it away. Under LSD, this "erasing" is slowed up, with the result that the former pattern persists, while the current pattern is being seen. This gives the viewer the strange perception that spots on a fern, letters on a page, or patterns on a wall, are "crawling" around. The effect is as if the temporal threshold for the phi phenomenon has changed; or perhaps there is a persistance

of an after-image effect. Whether such altered perceptions arouse fear or merely curiosity and interest must lie with the individual user and with the setting in which the perception takes place. A supportive social context tends to decrease fear and arouse interest.

Hofmann, the Swiss chemist who synthesized LSD and ingested a large amount of it, described his subjective experiences:

> My field of vision swayed before me and objects appeared distorted like images in curved mirrors. I had the impression of being unable to move from the spot, although my assistant told me afterwards that we had cycled at a good pace . . . As far as I remember, the following were the most outstanding symptoms: vertigo, visual disturbances; the faces of those around me appeared as grotesque colored masks . . . [I had] clear recognition of my condition, in which state I sometimes observed, in the manner of an independent, neutral observer, that I shouted insanely or babbled incoherent words. Occasionally I felt as if I were out of my body. . . . When I closed my eyes, an unending series of colorful, very realistic and fantastic images surged in upon me. A remarkable feature was the manner in which all acoustic perceptions (e.g. the noise of a passing car) were transformed into optical effects, every sound evoking a corresponding colored hallucination constantly changing in shape and color like pictures in a kaleidoscope. . . . I fell asleep and awoke next morning feeling perfectly well. (Hofmann, 1970)

Some investigators believe that LSD acts by being bound by the receptor proteins whose normal transmitter substrate is 5-hydroxy-tryptamine (Aghajanian and Haigler, 1974). 5-HT occurs in neurons of the raphe nuclei, a group of neurons of our brain stem. 5-HT was identified in these neurons by fluorescence methods (Dahlström and

Fuxe, 1964). Raphe nuclei neurons seem to be involved in inducing sleep, as well as other states of consciousness (Jouvet, 1965). These neurons have long axons that extend to all parts of our brain.

Nitrous oxide, like LSD, frequently produces a strange "detached" feeling that the subject is watching himself. "I felt as if I were far distant and that the individual performing the tests were someone else" (Steinberg, 1956, p. 190). LSD, psilocybin, and mescaline show cross-tolerance to each other. If you have been taking one of these three regularly, and try one of the other two, it takes a bigger dose to produce the same effect.

Marijuana, the crude leaves and flowers of the plant Cannabis sativa, contains a resin which includes a mixture of about eight cannabinoids, of which ℓ-Δ^9-tetrahydrocannabinol (Δ^9-THC) is thought to be the most psychoactive ingredient.

> The mechanism of action of Δ^9-THC is unknown. . . . Patients maintained on lithium or methadone continue to experience the effects of marijuana without apparent alteration. . . . Substantial behavioral effects of Δ^9-THC are seen with [low] doses that have no effects on brain 5-HT [5-hydroxy-tryptamine]. (Jaffe, 1975, p. 307)

The euphoria and psychedelia brought about by marijuana is, of course, different for different people. Anxiety is lowered. Light-heartedness is perhaps an apt term for this euphoric state. Friends may giggle over silly word puns. Thoughts rising to the surface of

consciousness may be more readily spoken, with less self-consciousness and self-censorship. Time perception is altered. Fresh ideas may appear and seem highly significant. One's mind may for the first time juxtapose two different bits of knowledge and see the relationship between them with sudden insight, and the perceiver may even wish to write down these new "profound thoughts." Short term memory of the words of a conversation, even the words one is speaking in a long or complex sentence, may drift away, perhaps interfered with by the sparkling rapid influx of new thoughts.

The last category of drugs which nitrous oxide falls into is that of the spindle poisons: nitrous oxide, colchicine, vinblastine, podo-phyllotoxin, and griseofulvin. Except for N_2O, these are many-ringed, complex molecules. A spindle poison is a substance that halts mitosis, not by denying the cell oxygen, ATP, or some essential metabolite, but by interfering with the normal building or function of the "spindle," that array of microtubules which holds and moves the chromosomes.

Colchicine is an alkaloid found in the autumn crocus or meadow saffron. Colchicine is used medicinally for the relief of gout. It inhibits the migration of granulocytes (a type of white blood cell) to the affected joint, and thus prevents the inflammation which causes part of the pain. Gout seems to be caused by deposits

of monosodium urate crystals that are deposited at the joint. Granulocytes come there to engulf these urate crystals. Colchicine is not an analgesic for any other type of pain. Colchicine is concentrated in kidney, spleen, and liver, but does not enter brain tissue.

Vinblastine, along with three other vinca alkaloids, is obtained from the periwinkle plant Vinca rosea, which is a kind of myrtle. Vinblastine binds onto tubulin, and dissolves microtubules, and forms crystals of tubulin-vinblastine in the cell. The binding site differs from that of colchicine and podo-phyllotoxin. Vinblastine is used in chemotherapy for cancer. While under treatment, leukemia may develop in the central nervous system. Vinblastine is thought not to cross the blood-brain barrier in any great quantity, but the patient may be depressed after a few days of treatment.

Griseofulvin is a mold metabolite, isolated from Penicillin. It is used medically against certain fungus infections. It is tightly bound to keratin, so that new hair and nails are free of the fungus infection, after administration. It may cause mental confusion.

CHAPTER VIII

THE ACTION OF ANESTHETICS AND ANALGESICS ON NEURONS

SPECIAL ATTRIBUTES OF NEURONS

Neurons are living cells that possess extraordinary attributes. They are complicated and highly organized, with intricate internal structures. Each part, such as the nucleus, the excitable cell membrane, the ribosomes, the mitochondria, the endoplasmic reticulum, the centrioles, and the microtubules, appears to have a special purpose or function. Even the individual proteins also have specific functions.

When considering where and how an anesthetic and analgesic like nitrous oxide might affect the use of oxygen within the neuron, a natural first place to look is at the process of "burning" sugar for energy by using oxygen. A human brain weighs less than 2% of our total weight. It uses 20% of both the oxygen we breathe and of the starches we eat. Starches are broken down into individual glucose molecules, and both oxygen and glucose cross over capillary walls to enter neurons. Our brains contain about 14 billion neurons (White, Handler, and Smith, 1973).

Inside the watery cytoplasm of the neuron cell, a series of cooperating proteins cleave the 6-carbon

sugar, glucose, into two 3-carbon pyruvate molecules. The first one of these proteins, hexokinase, is 20 times as abundant in neurons as in any other kind of cell. These cooperating proteins, the glycolytic enzymes, are located at axon terminals as well as in the central cell body. The third glycolytic protein in this series, phosphofructokinase, speeds up or slows down the whole process according to the needs of the neuron cell for ATP.

Blood glucose levels are normally at 80 mg/100 ml. In insulin coma, blood glucose levels have fallen to about 8 mg/100 ml. The mitochondria within the neuron cells cannot make enough ATP for the human to remain conscious. In normal health, 10% of the glucose molecules are used to make amino acids, lipids, and nucleic acids. Other tissues than brain do not convert so much glucose into protein.

Neuron cells transport amino acids from the blood into the neuron cytoplasm. These amino acids, and those made by the neuron proteins from glucose, are rapidly made into proteins. Protein synthesis occurs in the ribosomes, huge groups of about 80 cooperating proteins. Ribosomes are chiefly visible attached to the endoplasmic reticulum in the cell body, but protein synthesis also occurs in the nucleus, in the mitochondria, in the axon, and near the synapse (White, Handler, and Smith, 1973).

Neurons have the highest amount of RNA in the body,

reflecting their high rate of protein synthesis. Ribosomes contain about 50% protein and 50% ribosomal RNA. Every amino acid used in protein synthesis is first attached to transfer RNA. The printed message, designating the ordered sequence of amino acids for each kind of protein, is contained in messenger RNA. When a neuron is stimulated frequently, it synthesizes more protein and also more RNA.

PSYCHOLOGICAL EFFECTS FROM METABOLIC DEFICIENCIES

Both DNA and RNA are built of purines (adenine and guanine) and pyrimidines (uracil, thymine, and cytosine). Brain cells cannot synthesize pyrimidines "from scratch," but they can synthesize purines. Normally guanine is attached by a protein to the phosphorylated sugar ribose, and then this structure can be incorporated into RNA. A genetic defect, which is linked to the X-chromosome, causes this transferase protein to be almost completely missing (1% of the normal), and the behavioral result is mental deficiency, aggressive behavior, and self-mutilation (White, Handler, and Smith, 1973).

I have emphasized the utilization of glucose and oxygen to make ATP, and the high rate of protein synthesis in neurons, in order to draw attention to the maintenance of consciousness and the process of information storage into memories. Neuron membranes are excitable, keep sodium

ions outside, demonstrate an action potential when stimulated, and can transmit 500 impulses per second. We are able to measure these changes, and therefore consider them to be of primary importance. I consider the constant high biosynthesis of proteins to be of equal importance, if not more so. More than half of the soluble proteins of developing brains consist of the tubulins, in microtubules, and of dynein, in microfilaments (Olmsted and Borisy, 1973). Microtubules are constantly formed and dissociated in neurons (Inoué, 1953); this is at least as fundamental a process as the production of the action potential.

We have followed oxygen from lungs to hemoglobin, noting alterations produced by N_2O along the way. We have followed glucose into the neuron, and as far along as its change into two molecules of pyruvate. This process, anaerobic glycolysis, is not depressed by 95% N_2O (DiFazio et al., 1969). Pyruvate, a 3-carbon sugar, enters the mitochondria, and here one of the carbons is removed as carbon dioxide, while the other two carbons continue onward as acetyl-CoA. The complex of cooperating proteins which accomplish this step from pyruvate to acetyl-CoA, use the vitamin thiamine in the process. Thiamine deficiency shows in neurological signs: there is marked peripheral neuritis; deep reflexes are lost; sensory changes occur; there is anxiety and mental confusion. These symptoms

appear in chronic alcoholics as the Wernicke syndrome.

CONSCIOUSNESS, MITOCHONDRIA, AND ATP

The last 2 carbons, on acetyl-CoA, enter the Krebs cycle within the watery matrix of the mitochondria. Again a series of cooperating proteins accomplish the orderly removal of electrons and carbon atoms. The carbon atoms leave as carbon dioxide, and the electrons are transferred to a series of cooperating proteins which are attached to the lipid inner membrane of the mitochondria. These lipid proteins, the electron transport chain, use the energy from the moving electrons to make ATP, the energy unit for all living things. The electrons are transferred to oxygen, the final electron acceptor.

Maintaining consciousness and even life itself depends upon this production of ATP within the mitochondria. If oxygen cannot get to the mitochondria, we fall unconscious. If glucose levels fall, or if glucose cannot enter the neuron, we approach a diabetic coma. If a drug, such as the barbiturates, interferes with the functioning of the electron transport chain, our ATP levels fall, and we become drowsy (phenobarbital), go to sleep (seconal), or become unconscious (pentothal). ATP production by mitochondria cannot fall even to 97% of normal, without our becoming unconscious.

NITROUS OXIDE EFFECTS UPON OXYGEN USE

Nitrous oxide has been shown to produce a significant reduction in oxygen consumption in vivo (Schatte and Bennett, 1973; Schatte et al., 1974; Bradley, 1976). However, when DiFazio et al. (1969) measured oxygen consumption in vitro, using rat bone marrow in a Warburg flask, there was no significant reduction in O_2 consumed in 80% N_2O compared to controls. Nahrwold and Cohen (1973) isolated mitochondria from rat liver, and used "a polarographic estimate of state 3 oxygen uptake at 25°C with glutamate as substrate." N_2O at 67% caused a 5% inhibition of state 3 oxygen uptake, not significantly ($p > 0.10$) different from control values. Nitrous oxide in this experiment did enhance the effect of halothane upon O_2 uptake of mitochondria, producing a similar inhibition with halothane at 0.37% (N_2O at 67%) as was shown with halothane alone at 0.69%. Nahrwold related inhibition of oxygen uptake to lipid solubility (oil/gas partition coefficients) of the gas involved.

THEORIES OF ANESTHETIC ACTION

1. Lipid Solubility

The lipid solubility theory of anesthetic mechanism of action is one of the oldest theories (Meyer, 1899). Butler (1950) has rightly pointed out that these theories

depend on correlations of lipid solubility with anesthetic potency, but such correlations are misleading. Usually the authors have only examined a small group of drugs, often only a homologous series. The barbiturates form such a series, but since the discovery of the site of action of barbiturates upon the electron transport chain (Krebs, 1961), we no longer cite lipid solubility as an explanation for barbiturates. The opioid alkaloids form such a homologous series, but since the discovery of the enkephalins (Hughes, 1974), and the endorphins (Li and Chung, 1976), we recognize that the site of action of the opiate alkaloids is upon the protein receptor for our endogenous "opiates." Lipid solubility was not the explanation. The alcohols form such a homologous series. So do the anesthetics. One can form a series correlating anesthetic activity with lipid solubility for such chemically diverse drugs as ether, chloroform, paraldehyde, the barbiturates and trichloroethanol, the metabolite of chloral hydrate.

Lipid solubility theories have even been attempted for the inert gases, 1) hydrogen, 2) helium, 3) nitrogen, 4) neon, 5) argon, 6) krypton, 7) xenon, 8) ethylene, 9) cyclopropane, and 10) nitrous oxide. Helium does not produce narcosis or anesthesia. It is just as important to explain why a particular inert gas does <u>not</u> affect lipid systems as it is to explain why another gas does

so. Nitrogen at 10 atmospheres produces narcosis but not anesthesia. At this pressure the human body contains about 0.007 moles of nitrogen per kilogram. With ether under light anesthesia the body contains about 0.015 moles of ether per kg. Hydrogen gas produces no effect at 10 atmospheres, but causes loss of righting reflex in mice at about 130 atmospheres (Brauer, 1970). Neon has not been investigated. Because of the puzzling exceptions of helium, hydrogen, and neon, Case and Haldane (1941) abandoned the lipid solubility theory for inert gases.

Lipid solubility theories do not die; they merely expand to include more of the parameters of the inert gases that we are able to measure. Carpenter (1954) measured action potentials of isolated sciatic nerves from rats, using eleven inert gases, and plotted the atmospheric pressure needed for narcosis against the lipid solubility of these gases. This blockade of the action potential by 90% N_2O did not occur at 5 atmospheres, although oxygen consumption fell 35%. At 13 atmospheres 90% N_2O blocked the action potential, and oxygen consumption fell 65%, but this blockade took 20-30 minutes to be complete. Carpenter saw this as evidence of physical saturation of the nerve membrane lipids. This blockade was reversible. Carpenter pointed out that mitochondria make up about 50% of the total phospholipids in the central nervous system.

2. Hydrate Microcrystals

In 1961 Linus Pauling moved away from lipid solubility theories to advocate a theory of hydrate microcrystals which inclose a molecule of the inert gas in a lattice of water molecules. Hydrates can be formed with argon, krypton, xenon, methane, nitrogen, and nitrous oxide (deForcrand, 1902). Stability of the hydrate increases with the polarizability of the entrapped gas. The xenon hydrate is composed of one atom of xenon and 46 water molecules. Pauling suggested that the formation of hydrate microcrystals involves both the anesthetic agent and the side chains of amino acids. These side chains may exert a stabilizing effect, and allow the complicated hydrate to slowly form. Pauling suggested that such microcrystals may adhere to the cell membranes of neurons, and clog up the free movement of substances in the synaptic cleft. The resultant decrease in neuron firing is what we experience as narcosis, a loss of sensation accompanied by stupor. The formation of hydrate microcrystals undergoes a sudden change in phase from liquid to crystal.

Pauling's theory does not offer an explanation for the initial period of excitement that precedes the surgical anesthesia. It does not offer an explanation for the differences in induction times among the various anesthetics. Ether and methoxyflurane have long induction times, measurable in terms of their high blood/gas

partition coefficients, 15.6 for ether and 13.0 for methoxyflurane. Cyclopropane, at 0.46 and nitrous oxide at 0.47 have very fast induction times. Pauling suggested that even ether, which enters lipid membranes until there are 65,000 ml of ether in every 1,000 ml of lipid, does not alter the insulating properties of the neuronal membrane.

Ether, as well as nitrous oxide, has produced lowered production of white blood cells, as have xenon, cyclopropane, and ethylene (Pittinger et al., 1953). Nitrous oxide, ether, and cyclopropane all produce characteristic EEG changes correlated with depth of anesthesia (Courtin et al., 1950; Faulconer, 1952). However, with xenon in monkeys, even at three atmospheres, there is no burst suppression (Pittinger et al., 1955). Xenon produces anesthesia in humans even at atmospheric pressure. For other mammals, elevated pressures must be used. Xenon is often compared to N_2O, ethylene, and cyclopropane in its biological inertness. Featherstone and Muehlbaecher (1963) in a review on properties of inert gases used in anesthesia, rejected the idea that xenon or N_2O may inhibit glucose or pyruvate oxidation, or that these gases may interfere with the production of ATP by the electron transport chain.

3. Polarizability, Surface Film Affinity, Mole Volume, and Dipole Moment

The rare gases, helium, neon, argon, krypton, and xenon are simply single atoms which have a dense positive nucleus surrounded by a diffuse cloud of electrons. Centers of positive and negative charge coincide, and these atoms have no dipole moment. These atoms can, however, be polarized; that is, in the presence of a charged molecule, their electric symmetry is distorted, the electron cloud being drawn toward a + charge, or repulsed by a - charge. This polarization is an induced dipole moment. The heavier rare gases, such as xenon, are more easily polarized than the lighter ones, such as helium or neon. Nitrous oxide (3.2) can also be polarized, with a value falling between krypton (2.46) and xenon (4.00). These numbers are an index of ease of polarization.

A measure of the dipole moment, in debyes, is given by the tendency for an electric current to flow through a gas, as compared to the flow through a vacuum. The dipole moment unit is a product of the magnitude of the charges and the distance separating the charges. One debye unit = 1×10^{-9} electrostatic units X n m. Nitrous oxide by itself has a dipole moment of 0.167 debyes, small compared to water (1.85 debyes), but still differing from carbon dioxide, CO_2 (0.000 debyes).

Ostergren, in 1944, suggested that changes in mitosis

and narcosis are both brought about by a single mechanism, interaction of the chemically unreactive gas, N_2O, with the lipophilic side chains of proteins so as to cause a change in the shape of the protein molecule. It is a mark of the brilliance of Ostergren that, even without an electron microscope, or a sight of the action of centrioles and microtubules, he still was able to hypothesize the essential role of nitrous oxide in affecting both mitosis and consciousness.

Lipid solubility of nitrous oxide was suggested as the causative factor of narcosis by Sears and Fenn (1957) using an oil-in-water emulsion and N_2O at 53 atmospheres. Lipid solubility of N_2O was investigated by Clements and Wilson (1962), using a monomolecular film of several membrane lipids, and noting surface tension changes. A square cm of film absorbed 0.16×10^{-10} mole of N_2O. Correlations were made for eleven anesthetic agents between anesthetic potency and surface film affinity.

Featherstone and Muehlbaecher (1963) plotted a number of measurable parameters of gaseous anesthetics against their anesthetic potency. Going on the assumption that inert gases, such as cyclopropane and N_2O, produce anesthesia in the same manner as xenon, they considered both polarizability and mole volume, or Van der Waals b factor, the volume which 6.02×10^{23} molecules of a

gas occupied at the critical temperature and pressure. This measure refers to the finite volume of molecules, and to their general incompressibility. For N_2O this mole volume is 4.42×10^{-2} liters/mole, between krypton (3.97) and xenon (5.08). They believed that the ratio of polarizability to mole volume must be the critical measure to correlate with anesthetic potency. This ratio for N_2O is given as 0.92, bigger than xenon, 0.82.

Lipid solubility can be thought of as a measure of the Van der Waals attraction between a gas and the lipid molecules. Such forces are at 0.5 K cal bond strength compared to 2-5 K cal for hydrogen bonds, and 5 K cal for ionic bonds. Absorption of a gas at a membrane, an interface, or in a lipid "pocket" of a protein is also held by these Van der Waals forces. Vapor pressure of a gas, often used in thermodynamic activity theories of narcosis, depends on the Van der Waals attraction of the gas molecules for each other. Pauling's hydrate microcrystals depend on Van der Waals forces attracting the water molecules to the gas. Featherstone and Muehlbaecher discount the permanent dipole moment possessed by N_2O (a value of 0.167 debyes) as a factor, because xenon has no permanent dipole.

4. Cation Permeability and Pressure Reversal

These same measures on the lipid solubility of N_2O, its van der Waals forces, polarizability, molecular volume

and permanent dipole, are cited by Gottlieb et al. (1968) in an experiment on sodium active transport. This experiment was performed on frog skin in Ringer's solution, using a pressure of 200 pounds per square inch. N_2O was compared to krypton and xenon. N_2O (200 psig), xenon (200 psig), and krypton (950 psig) showed marked inhibition of Na^+ active transport as measured by short circuit current techniques. However, no effect from N_2O was observed during the first hour, even at 200 psig.

Brauer (1970) compared nitrous oxide with hydrogen, helium, and nitrogen, on the effect of these gases under pressure, upon the loss of the righting reflex in mice. Helium did not alter the righting reflex, instead producing convulsions at 85 atmospheres. Hydrogen produced loss of righting reflex at 128 atmospheres. Nitrogen produced loss of righting reflex at 32 atmospheres. N_2O produced loss of righting reflex at 1.3 atmospheres. Helium and hydrogen were the "weakest" in anesthetic effects, nitrogen the next weakest. Brauer mentions that argon is about twice as potent as nitrogen, and that xenon is slightly more potent than N_2O. N_2O seems to be 25 or 28 times as potent as nitrogen. Krypton is about 10 times as potent as nitrogen. Brauer (1970), like Featherstone and Muehlbaecher (1963), correlated narcotic potency with the ratio of polarizability to molal volume. Brauer proposed a model for inert gas narcosis which involves the reversible

formation of some compound involving the inert gas and "specific reactive sites in the susceptible tissue" (Brauer, 1970, p. 29).

Roth et al. (1976) returned to the lipid solubility theory in an experiment on frog sciatic nerve in Ringers solution, measuring depression of action potential amplitude by N_2O under pressure. He hypothesized that anesthetics expand the lipid component of membrane. Action potential amplitude was depressed 50% by 4.86 atmospheres of N_2O, and depressed to 10% of control value by 8.2 atmospheres N_2O. When helium was added to the N_2O to increase the pressure to 68 atmospheres, the action potential amplitude rose again to 70% of control values. The increase in pressure seemed to reverse the anesthesia. Roth speculates on the higher concentration of anesthetic needed to depress transmission in axons, compared to the lower concentration needed to block synaptic transmission. He feels this indicates the factors of fiber size and myelination are crucial. Volume expansion of lipid membranes is implied by the puzzling phenomenon of pressure reversal of anesthesia.

This offspring of the lipid solubility theory has been termed the critical volume hypothesis. This hypothesis suggests that the lipids absorb an inert substance such as N_2O until they expand beyond a certain critical volume, and then anesthesia occurs. Such a hypothesis emphasizes

the role of lipids in the neuron membrane; it is equally important to emphasize the lipid pockets in microtubular proteins, since we can see the changes in these proteins under N_2O. Pressure opposes anesthesia by compressing the lipids. Johnson (1973) prepared artificial phospholipid membranes in a single spherical bilayer shell of 24 nm to 50 nm diameter, surrounding an aqueous center. Cation permeability was measured for these vesicles with different anesthetics. Cations are positively charged ions, such as H^+ (hydrogen), Na^+ (sodium), K^+ (potassium), Mg^{++} (magnesium), and Ca^{++} (calcium). Neurons and other cells spend energy (ATP) to keep Na^+ outside the cell membrane, and keep K^+ inside the cell membrane. Inside a relaxed muscle cell Ca^{++} ions are segregated in the sarcoplasmic reticulum, a double membrane "lace sleeve" surrounding each bundle of myofibrils. When a nerve impulse stimulates the muscle cell, this sarcoplasmic reticulum double membrane increases in permeability, and the Ca^{++} ions leak out in huge numbers, causing the muscle to contract. When the muscle is no longer stimulated by the nerve impulse, the sarcoplasmic reticulum membrane proteins pump the Ca^{++} ions back inside the membrane, using energy (ATP) to do so.

Mg^{++} (magnesium) is required by many proteins and is often held by ATP or ADP (the unenergized form). H^+ ions are used by mitochondria in forming ATP as electrons

flow along the electron transport chain. Mitochondria keep H^+ ions outside their inner membrane and use this electrical (more acid) gradient to help in making ATP.

Thus it is obvious that if anesthetics such as N_2O disturb this careful segregation of cations by the membrane proteins, this might go far to explain their effect. Neurons carry an impulse along the axon by the action potential, a sudden leaking in of Na^+ ions which changes the inside electrical potential from -70 mv to +40 mv. Neurons recover the -70 mv potential by first letting K^+ ions outside, and then pumping out Na^+ ions, using ATP in the process.

Johnson (1973) measured cation permeability by forming the spherical membranous liposomes in an aqueous solution containing $^{42}K^+$, radioactive potassium, and/or ^{14}C glucose, radioactive glucose. When the membrane was rendered more permeable by the anesthetic, the escaping radioactivity could be measured in a scintillation counter. ^{42}K could be counted at once; ^{42}K decays completely in 10 days, and then the radioactive glucose could be counted. Johnson plotted the permeability of potassium against increasing concentrations of ether, nitrous oxide, helium, halothane, chloroform, and sodium pentobarbital. As a dosage level for these anesthetics, he chose that concentration which produced loss of righting reflex in the newt. N_2O was used at about 6 atmospheres, 11 atmospheres, and 16

atmospheres. Newt anesthetic pressure actually occurred at 0.75 atmospheres. Helium is not an anesthetic gas, and was used in order to increase pressure only, with the result that less ^{42}K leaked out of the membranous liposomes, even in the presence of an anesthetic dose of ether, as helium pressure was raised to 100, 200, and 300 atmospheres. This pressure reversal also applied to halothane, chloroform, and sodium pentobarbital.

The anesthetics produced an increase in membrane volume, and a disordering of the bilayer structure. Glucose permeability was not affected by the anesthetics. Pressure might squeeze out "molecules of the anesthetic from the membrane, or pressure may simply compress and re-order the membrane." Johnson (1973) favors this latter hypothesis, partly because this gives him a completely self-consistent argument.

In reviewing the effects of N_2O upon the cell, particularly the neuron, I have discussed N_2O effects upon glycolysis, upon use of oxygen in vivo and in vitro, and upon mitochondria. I have reviewed lipid solubility theories of narcosis by nitrous oxide and its effects upon the action potential. I have reviewed hydrate microcrystal theories of narcosis by N_2O, and theories considering its polarizability, molal volume, and dipole moment. Experiments which have used as a dependent variable the loss of righting reflex notice an effect from N_2O

at atmospheric pressure or close to it (1.3 atm, Brauer, 1970). Experiments which use as a dependent variable a loss of the action potential, or leakage of K^+ ions, notice an effect from N_2O only at 4.86 atmospheres (Roth, 1972), at 6 atmospheres (Johnson, 1973), at 13 atmospheres (Carpenter, 1954), or at 200 psig after one hour (Gottlieb, 1968).

ANALGESIA AND ENDOGENOUS "OPIATES"

The use of analgesia as the dependent variable to measure the effect of nitrous oxide has brought to light some puzzling interactions with other drugs. Ever since the discovery of our endogenous "opiates," enkephalin (Hughes, 1975) and β-endorphin (Li and Chung, 1976), experimenters have attempted to explain other analgesic effects through these same endogenous "opiates." It has been shown (Kuhar et al., 1973) that morphine and other opioid alkaloids are bound by certain specific opiate receptor proteins, which exist in a wide variety of areas in the brain.

The perception of pain is a curious phenomenon that involves many different levels of analysis. Emotions such as fear or despair can magnify the affective aspect of pain. Joy or excitement can diminish this affective aspect so much that the person may not even notice an injury at the time. Analgesics such as morphine, heroin,

demerol, fentanyl, and nitrous oxide relieve pain in ways that we are just beginning to understand.

This subjective experience of pain seems to be lacking in some people (McMurray, 1950; Baxter, 1960). To be born with no pain sense would seem to be a blessing, but such children are in grave danger of doing themselves some permanent injury. A three-year-old boy liked to thrust his hands into the flames; "it tickles," he would respond when asked why he did it. Such individuals may, with care, live to be 35 or 40 years old, when death occurs from bone disease brought on by not moving while they sleep.

BRAIN DISTRIBUTION OF OPIATE RECEPTORS

Normal pain sensation, from arms, legs, and body, travels up the spinal cord to a "way station" in the lower brain stem, called the substantia gelatinosa. Evidence that these particular neurons are involved in pain sensation, and in the inhibition of pain sensation, comes from an experiment performed by Pert, Kuhar, and Snyder in 1975. Rats were injected i.v. with radioactively labeled ^{3}H-diprenorphine, an extremely potent morphine antagonist. After one hour, the rats were killed, the brains cut into 4 mm coronal sections, frozen in liquid nitrogen, cut to 4 μm thickness, and put on emulsion coated microscope slides. After five weeks of exposure in a dark lead-lined

cabinet, the slides were developed, stained, and viewed. Autoradiographic grain counts were made of 2,400 μm^2 areas for each region of the brain. Mean number of grains per 100 μm^2 showed very different distributions for the different areas of the brain. The substantia gelatinosa in the spinal cord or lower brain stem showed 10.4 grains per 100 μm^2.

Opiate receptors are highly selective proteins that occur in high concentrations in the post synaptic membranes of specific neurons in certain well defined regions of the brain. After the pain sensation has gone through the substantia gelatinosa, it moves upward through the locus coeruleus, a small group of neurons scattered through the reticular formation, that part of our brain involved in sleep or arousal. Here opiate receptors were found labeled with 10.0 autoradiographic grains per 100 μm^2. These neurons of the locus coeruleus are thought to be involved in the response to anesthetics such as ether, halothane, or methoxyflurane.

Above the reticular formation lies the substantia nigra, where a second group of neurons, those of the zona compacta, also possess opiate receptors and thus also seem either to allow the pain stimulus to proceed onward or to inhibit this message. Autoradiographic grains in this group, the zona compacta of the substantia nigra, were 6.5 grains per 100 μm^2.

Traveling upward from the substantia nigra, we come, in mammals, to the thalamus. Here a curious anomaly presents itself, understandable only in terms of evolution. Only mammals have a thalamus, that central, two lobed body of neurons that seems to us to be the center of our consciousness, our thoughts, emotions, and feelings. The more primitive structure--the striatum--fulfills in all other vertebrates, who are not mammals, all the functions that the thalamus fulfills in ourselves. We also have a striatum, which has been pushed outward to both sides, as the thalamus developed in the most central position. Our sense of smell, with its emotion-laden nostalgic associations, comes directly from nose to striatum, and thence around to our amygdala, those "tails" of the striatum that extend into our temporal lobes. Autoradiographic grains labeling the opiate receptors of the striatum showed the highest concentrations in the brain, 11.5 grains/100 μm^2 in the nuclear "streak" of the caudatus putamen, 10.5 grains in the nuclear clusters of the caudatus putamen, and 12.3 grains/100 μm^2 in the amygdala. Here then in our striatum, including the amygdala, is our greatest concentration of opiate receptors; both pain and pleasure seem to enter awareness through these brain areas.

Our thalamus, hypothalamus, and hippocampus form a connected unit through the fornix. The fornix, twin bundles of axons from the hypothalamus, stretches upward,

backward, and around into the temporal lobes, ending in the hippocampus which lies just beside the amygdala. Thalamus sections contained 5.5 grains/100 μm^2, hypothalamus contained 6.6 grains and hippocampus contained 1.7 grains/ 100 μm^2. The amygdala, with 12.3 grains, contrasts sharply with its closest neighboring structure, the hippocampus, both of these lying in the temporal lobes.

The grey cell bodies of the cerebral cortex are in general separated from all these central structures of our brain (thalamus, striatum, etc.) by masses of axons which appear white because of their entwining myelin sheaths. The only place where grey cell bodies of the cortex merge closely with grey cell bodies of the central structure is the septum, a part of the frontal lobe cortex which touches the hypothalamus, and is indeed continuous with it. Here is another opiate binding area of the brain, forward from the hypothalamus through the septum and on forward to the frontal pole of the cerebral hemispheres. Opiate binding assays for frontal pole were performed on human brains with dihydromorphine by Goldstein et al. (1971); results were 14.6 f mol (per mg protein).

Human brains, monkeys, rats--it does not surprise us if mammals like ourselves possess opiate receptors. But all vertebrates, even the most primitive fish, have as many opiate receptors as we do. Invertebrates do not have opiate receptors.

ATTEMPTS TO RELATE OPIATE RECEPTORS TO BRAIN TRANSMITTERS

Researchers have attempted to correlate those neurons which have opiate receptors with particular neurotransmitters used. Morphine and other opiates inhibit the firing of single cerebral cortex cells. Perhaps our endogenous enkephalins or endorphins act as inhibitory transmitters. Kuhar, Pert, and Snyder (1973) compared regional opiate binding to brain distribution of choline acetyl transferase, gamma aminobutyric acid, and tyrosine hydroxylase in monkey brains. None of these substances showed correlation with opiate receptor binding.

Another method of attempting to trace opiate binding receptors which correlate with a particular neurotransmitter is by destroying a particular area with an electrolytic lesion. For example, lesions in the septal area of the rat brain, near the fornix, destroy cholinergic projections to the hippocampus. Four days later, or 15 days later, choline acetyltransferase levels have fallen from 3.9 μmol/hour/gram to 0.65 μmol/hour/gram, but opiate receptor binding remains at the same level, 19 f mol per mg protein before and after lesioning (Kuhar et al., 1973).

Lesions of the locus coeruleus destroyed ascending norepinephrine axons in the ipsilateral cerebral cortex, and NE levels there fell 70%. But opiate receptor binding remained constant 21 days later at 4 f mol per mg protein.

Destruction of the raphe nuclei of the pons decreases 5-hydroxy-tryptamine levels in the forebrain of rats by 75% after 15 days, but opiate receptor binding levels showed no change from 2 f mol per mg protein.

These results are puzzling. How do our endogenous opiates act? They seem to be unrelated to any known neurotransmitter system.

Addiction is an aspect of opiate use which may shed light on the effects of endogenous opiates. Addiction includes tolerance, physiological dependence, and compulsive craving. Tolerance implies we need a bigger dose to get the same effect. Physiological dependence involves the appearance of withdrawal symptoms. Compulsive craving is a term used to describe wanting a drug again even many years later, when withdrawal would presumably be long past.

Opiate receptor assays do not show any systemic changes in addiction. Opiate agonists do, however, decrease cAMP in a clone of neuroblastoma-glioma hybrid cell culture and increase cGMP (Klee, 1974; Sharma, 1974; Traber, 1974). Prostaglandin E_1 and adenine normally stimulate adenylate cyclase; morphine antagonizes this normal stimulation (Klee, 1974). Cells that are "tolerant" to morphine no longer show this antagonism (Klee, 1975). Cells put into "withdrawal" by naloxone become supersensitive to prostaglandin E_1 as a stimulator of adenylate cyclase.

BRAIN ELECTRICAL STIMULATION AND ENDOGENOUS "OPIATES"

Endogenous opiates seem to be released by electrical stimulation of brain sites surrounding the cerebral aqueduct and the III and IV ventricles (Mayer, 1975). This stimulation-produced analgesia can be produced by trains of biphasic, rectangular wave pulse-pairs: 50 μsec of +7 milliamperes, 100 m sec rest, 50 μsec of -7 milliamperes, 100 m sec rest and so on. This stimulation frequency was generally 20 per second. Brain stimulation is applied for 20 seconds before testing for analgesia. The stimulation intensity required for analgesia has to be individually found for each animal, and varies from 1 to 7 milliamperes. These individual differences are also seen in analgesic drug requirements for humans. For some people 23% nitrous oxide is already an overwhelming dose; for others, 50% nitrous oxide does not yet affect them.

After rats showed reliable analgesia from 20 seconds of brain stimulation, they were tested to see if tolerance developed. Control points of 85% to 95% analgesia showed reliability when tested at two week intervals (Mayer, 1975). If the rat were stimulated for 24 hours, 10 pulses per second, of the 200 μsec pulse, and rested for 24 hours, then given the regular 20 second brain-stimulation and then tested for analgesia, then percent of analgesia had

fallen to 73%. Another 24 hours of rest, followed by 24 hours of brain stimulation, then 24 hours of rest preceded the second testing for tolerance. Again, 20 seconds of brain stimulation, which normally evokes about 90% analgesia, now evoked only 47% analgesia. Tolerance did not increase beyond this point. Recovery of analgesia occurred in two weeks.

Cross-tolerance to morphine was shown (Mayer, 1975). First, rats were shown to exhibit reliable analgesia with brain stimulation. Then they were injected twice daily with increasing doses of morphine sulfate: up to a high dose of 600 mg/kg per day. One day after this last dose of morphine, analgesia had fallen from 85% to 53%. A further drop to 44% analgesia occurred the following day. Analgesia was tested for by giving brain electrical stimulation just 20 seconds before the hot plate tail-flick test. There was no change in baseline latency behavior (response to radiant heat within 4 seconds when no brain stimulation was applied), showing that normal response to painful stimuli was not affected by withdrawal from morphine. Recovery from this cross-tolerance to morphine occurred from two to four weeks later.

Analgesia produced by brain stimulation can be prevented by the administration of naloxone, a morphine antagonist (Akil et al., 1976). In this experiment the rectangular biphasic pulse-pairs of brain stimulation

were given at a rate of 3 per second (100 m sec each) for a period of 20 seconds. Current intensity was raised in steps of 10 μ amperes until 100% analgesia was reached. Naloxone, 1 mg/kg, reduced analgesia on the average to 62%, with some animals showing a reduction to 7%. Naloxone did not affect the baseline latencies, a measure of pain responsiveness without brain stimulation. Higher doses of naloxone (2 to 4 mg/kg) did not cause any further reduction in analgesia. Brain stimulation from electrodes placed in the periventricular grey matter produces analgesia in humans as well as in rats. Eight patients have been relieved of chronic pain for three years, by this method of analgesia.

CHAPTER IX

THE STRUCTURE AND FUNCTION OF MICROTUBULES IN CELLS

Whether we consider the nature of consciousness or whether we consider the effects of anesthetics and analgesics, or whether we consider the effects of nitrous oxide upon brain neuron groups, we always come to the point of focusing our attention upon the neuron cell itself.

Neuron cells are unique in many ways. They may have elongated processes which are 100,000 times as long as their diameter. They are able to produce a summed pre-synaptic dendritic potential, an action potential, and synaptic transmitter substances. In these ways they are highly specialized for communication. In other ways, neurons resemble the other cells of our body; in fact, neurons share some structures and functions with all other eukaryotic ("true nucleus") cells. One example of such a structural and functional process shared with other eukaryotes is the process of the growth and function of microtubules, a process which allows varied application to different tasks.

In order to understand how neurons function, there are two compelling reasons for studying microtubules.

One reason is that microtubules are strikingly involved in the processes of motility and sensitivity. Together, these are the two essential components of irritability or responsiveness, a universal property of living creatures. Mobility and sensitivity are equally important processes in behavior and experience, just as the processes of growth and responsiveness are in the functions of nervous tissue. A second reason for studying microtubules lies in their ability to form and reform at high rates. This ability suggests a process which can proceed in real time as a correlate of behavior and experience.

In this thesis, which attempts to show how nitrous oxide could produce its remarkable effects upon behavior and experience, the study of microtubules has a particular relevance. Nitrous oxide is one of a group of compounds, described in Chapter VII, which affect the spindle of a cell in the process of division. N_2O is a "spindle poison." Microtubules make up the spindles of dividing cells.

Neurons are the first cells to differentiate in the newly conceived human being. Before this differentiation occurs, egg and sperm have united and a large number of cell divisions have taken place. Cell division and mitosis are very complex processes. Human genes, like those of all other eukaryotic cells, lie along the human chromosomes in carefully specified, ordered segments

of DNA. DNA is deoxyribonucleic acid, and occurs in the 23 pairs of chromosomes which lie in the nucleus of every human cell. Each of our cells contains about 2 meters of DNA. There are about 100,000 different kinds of proteins, and the specification of every one of these proteins is encoded in its own specific segment of the DNA (Lehninger, 1975). Each of the 23 pairs of chromosomes contains one chromosome from the mother and one from the father. Presumably at the moment of conception a decision is made as to which gene will later find some phenotypic expression in the living creature, and which gene will be "silently" carried on to the next generation. However, in females, who have two XX chromosomes, one from the mother and one from the father's mother, the decision as to which X chromosome will be expressed is different in different cells of the body. Calico cats or pinto ponies have hair of different color on different patches of their skin, and this is due to one or the other of the XX chromosomes being expressed. The non-expressed chromosome can be seen in neuron cells, which also differ in this way, as a small dark body held against the nuclear membrane, the Barr body (Barr and Bertram, 1949).

When mitosis is about to begin, a complicated process takes place. Each strand of DNA in each chromosome must be carefully copied. The two copies must be separated and pulled to opposite poles of the cell. Eukaryotic

DNA does not exist as a naked helix of two strands of DNA, but as DNA wrapped in proteins. These chromosomal proteins, called histones, and non-histone chromosomal proteins, play some not-well-understood role in the decision as to which parent's gene is to be expressed. Of course, just as the whole two meters of DNA must be copied exactly, so must the chromosomal proteins also be duplicated and attached each to its own segment of DNA.

After the protein-wrapped DNA is duplicated, the doubled pairs of chromosomes appear as short, fat, dark bodies which are embedded in the spindle. The spindle, which is seen during mitosis, is constructed of microtubules, which connect the chromosomes in the center to the two poles of the spindle. At each pole of the animal cell spindle is a pair of centrioles. All eukaryotic cells, both plant and animal, have microtubules.

Each of these paired centrioles appears under the electron microscope as a cylinder; centrioles are always set at right angles to each other. A cross section of one centriole shows a flower-like arrangement of microtubules: nine microtubules arranged around a circle. Tenuous threads of protein form the spokes of the wheel. Cilia, flagella, mitotic spindles, axonal microtubules, and sensory processes are all subcellular structures built of microtubules and centrioles.

One of the pair of cylindrical centrioles seems

to act as the root: of a cilium, a flagellum, a sperm tail, one half of the mitotic spindle; of the outer processes of a rod cell in the retina; of the outer process of an olfactory cell in the nose; and so on. The other centriole is at right angles to the actively growing centriole, is very short, and appears to be passive until just before mitosis. When a eukaryotic animal cell is about to undergo mitosis, not only is the DNA doubled and carefully separated among the two daughter cells, but also the two mitotic centrioles. This doubling of the centrioles comes about in an organized, unidirectional process, which is repeated every time animal cells divide. First the longer centriole gets still longer, 5 to 10 times its pre-mitotic length. The short centriole doubles or triples in length. Both centrioles are still very close to each other at one end, almost touching, while their growing ends stretch out at right angles to each other, toward what will be the poles of the spindle. Now the two polar ends of the centrioles start to grow the microtubules that will form the spindle. As the microtubules form the spindle, the centrioles part from each other and each old centriole moves to one end of the spindle. At once a new small centriole is formed, at a right angle to, and next to the old centriole, from which stream the microtubules of the spindle. Thus the new daughter cells, when mitosis is completed, will each

have one old centriole and one new centriole; one daughter cell got the long cylinder, the other daughter cell got the short cylinder; each replicated the opposite kind to complete the pair.

Evidently some critical evolutionary advantage follows from this careful division, replication, and perpetuation of the centrioles (Pitelka, 1974). Animals supply all their cells with these identical centrioles. All cells are capable, then, of developing a cytoplasmic network of microtubules, useful in transporting proteins to the periphery of the cell, or from the periphery to the nucleus. For cells that will divide further, centrioles are able to produce the mitotic spindle. For cells that will produce cilia (such as olfactory neurons, "hair" cells of the cochlea, "hair" cells of the vestibular system that are involved with our sense of balance and head position, or cilia of our bronchial tubes), centrioles, after mitosis, will migrate toward the cell membrane and start to grow into the appropriate structure. "Both the orientation and the spacing of young centrioles, bearing microtubular rootlets, are anarchic at first" (Pitelka, 1974).

The organization of the centriole, and thus of the self-assembling microtubules which form the nine outer tubules of the circle, is both determined by pre-existing structures and yet also modifiable when local conditions

change (Pitelka, 1974). Microtubules are composed of two proteins, α-tubulin and β-tubulin, whose amino acid sequences, as with all other proteins, are encoded in our DNA (Olmsted and Borisy, 1973). A single microtubule is formed as a hollow cylinder of 13 tubulins in circumference, alternating α- and β-tubulins which form a helix as they build the cylinder lengthwise (Olmsted and Borisy, 1973). This basic assembly process seems to be determined (Pitelka, 1974).

Whether microtubules will attach themselves to chromosomes during mitosis or meiosis, what direction they will choose to build toward, whether they can pull the chromosomes toward the centrioles at the poles of the spindle--these behaviors are altered in the presence of nitrous oxide. Microtubules are also generally assumed to function in the transport of proteins from their assembly place, in the ribosomes of the endoplasmic reticulum, to their place of use: perhaps in the cell membrane, perhaps at the synapse, perhaps to be released from the cell as endorphins, oxytocin, vasopressin, or hypothalamic releasing factors. Besides functioning as transporters of protein from the nucleus toward the cell membrane, microtubules in neurons also transport proteins from the synapse back to the nucleus.

Microtubules function in white blood cells in several different ways. Mitosis is accomplished by microtubules

that form the spindle and the centrioles. Proteins are transported to and from the nucleus. Some white blood cells move among the red blood cells, or through capillary walls, much as an amoeba moves with its pseudopods. Microtubules form the structure of these pseudopods, thus acting as "muscles" for the mobile cell. Cells that form flagella or cilia, such as sperm or bronchial tube cells, do so with a centriole built of the nine outer microtubules and two more microfilaments made of dynein at the center of the circle (see figure in Appendix II). The two central microfilaments determine the plane of the beat in these motile structures. Sperm tail centrioles form a hollow cylinder near the body of the sperm, made up of 27 microtubules, three at each of the 9 places around the circle, as well as an extensive system of 9 branching roots that reach out to touch the surrounding mitochondria (see figure in Appendix II). A short distance away from the body of the sperm the two central microfilaments which are characteristic of cilia and flagella in eukaryotic cells start to form, and these extend the whole length of the sperm tail. These are not made of tubulin but of dynein, a different protein.

Some protozoa possess cilia or flagella. A few examples are Paramecium, Stentor, and Euglena. These tiny one-celled animals are eukaryotic cells, that possess a nucleus and undergo mitosis just as our own cells do.

Many microbiologists have remarked upon the responsiveness of these tiny creatures, comparable to that of higher animals in the purposive behaviors they exhibit.

Such responsiveness is in sharp contrast to the behavior of bacteria or plants. Bacteria are prokaryotic cells. They have no nucleus, no mitochondria, no chloroplasts, no protein wrapped around their DNA. They do not undergo mitosis. Cell division is accomplished by duplication of their single, circular, naked DNA, but no spindle is built. Bacteria often have cilia or flagella, but these are not built of tubulin, but of a bacterial protein flagellum. These cilia or flagella beat by means of a fixed machine-like arrangement of rings set in the stiff peptidoglycan cell wall. Bacteria with flagella may move when local conditions are not optimal for their survival and, by chance, may come to an area where conditions are more suitable. The directed, efficient responsiveness of single-celled eukaryotic cells is not a characteristic noted in bacteria by microbiologists.

Multicellular plants are also made up of eukaryotic cells. As a plant cell reaches its full size, it forms an outer cell wall of cellulose that is thick, strong, rigid, retaining, and protective. Such an armored device does not allow for maximum responsiveness or for further cell division and, indeed, there are no functional centrioles in these vegetative cells.

Tubulin, the protein in our own microtubules is the same protein in the mitotic spindle, in cilia, flagella, and in neurons, when judged by the criteria of molecular weight, amino acid composition, electrophoretic mobility, and immunological specificity. α-tubulin migrates more slowly, and β-tubulin migrates faster in an electrical field.

The centriole, with its 9 outer triplets, is inherently asymmetrical, and possesses polarity in its microtubular skeleton. The triplets are set at an angle to the radii of the centriole. The slanted angle of the triplets specifies directions both around the centriole and along its axis. Frequently one of the triplets will possess a "ribbon" of microtubules extending the transverse axis of this triplet. Such a microtubule ribbon distinguishes this triplet from all others, and thus also specifies the other eight triplets in a unique relationship to this "landmark" ribbon (Pitelka, 1974). The ribbon forms an index marker; the angled triplets specify a direction. Such an arrangement could form a useful geometric, spatial code which could supplement the linear code of our DNA.

Animal cells "migrate" during our embryonic development, thus differing in yet another way from plant development. Such cell migration is accomplished by microtubular pseudopods. Growth, the addition of new

protein at the tip of the growing neuron, involves protein transport by microtubules. Recognition of surrounding cells involves carrying a message back to the nucleus and microtubules can do this. Sensory processes, dendrites themselves, the most responsive and sensitive tissues, are all built by centrioles and microtubules.

Is it possible that not only responsiveness, but also memory, could be a function of microtubules? A union of memory with responsiveness produces learning. Perhaps this is the critical evolutionary advantage which is the function of centrioles in animal cells.

Neurons, of course, contain many other subcellular structures than microtubules and centrioles. A neuron is a delicately balanced symbiosis of thousands of proteins. I have spoken in this chapter of chromosomal proteins that enfold our DNA, and of the tubulins that make up our centrioles, first of all because of their early and striking motility in the process of development. If you watch a movie of the development of an embryo, these structures are the first actors whose motions are seen upon the microscope slide.

CHAPTER X

EFFECTS OF NITROUS OXIDE UPON MICROTUBULES

I have described in some detail the role played by microtubules and centrioles in the functions of cells, especially neurons. I shall now return to a more detailed consideration of how nitrous oxide produces its remarkable effects upon mitosis. Earlier in this thesis, under "Physiological Experimental Studies on N_2O," I described an experiment by Brinkley and Rao (1973), in which N_2O was shown to affect mitosis. If the N_2O treatment lasted for 4 hours, cells recovered and proceeded through mitosis. If N_2O treatment lasted for 12 or 16 hours, cells did not recover.

Microtubule proteins form the spindle which is visible during metaphase. This protein, tubulin, occurs in a dimer form, α-tubulin and β-tubulin. Tubulin attaches to the kinetochore region of the chromosomes, and the microtubules are built in an assembly process that uses GTP. α- and β-tubulin each have molecular weights of about 52,000. Two other unnamed proteins, that weigh 290,000 and 310,000, seem to participate in microtubule assembly. Microtubules seem to be assembled at a rate of about 5μ per minute. α- and β-tubulins differ in amino

acid composition and sequence, but the differing amino acids seem to have evolved through single nucleotide changes in the coding triplets. Thus, α- and β-tubulins may have evolved from a single ancestral protein, before the chordates diverged from the echinoderms. The primary structure of these crucial proteins was strongly conserved through millions of years of evolution (Snyder and McIntosh, 1976; Luduena and Woodward, 1973; Bryan, 1974).

All cells divide, from prokaryotes to humans. But prokaryotes do not have microtubules. Eukaryotic cells have microtubules and use them in mitosis, in changing their shapes, in cell movements, and in transport of proteins and other complex substances down the axons of neurons. Squid axoplasm contains dense strands of microtubules running lengthwise down the axon.

Tubulin is transported down the axon into the nerve ending in mammalian brain (Feit et al., 1971). Tubulin, like other proteins, is synthesized by ribosomes using a mRNA code, and this occurs in the cytoplasm of the cell body, not any great distance from the nucleus. Axons or dendrites may be more than a meter long in humans and even longer in larger animals. Some tubulin is transported very rapidly down the axon and some tubulin more slowly. Tubulin may be associated with membranes at the nerve ending.

Unlike colchicine, N_2O has no discernible effect

on the formation and assembly of spindle microtubules. Yet the chromosomes do not line up properly on the metaphase plate.

Brinkley and Rao (1973) observed this effect on Hela cells which had been partially synchronized by a thymidine blockade. After release from this block, the Hela cells were placed in fresh media in petri dishes, allowed to attach to the dish, given a change of medium, and placed in the nitrous oxide chamber under pressure (80 lb/in^2 at 37°C). A control batch of cells, also synchronized in S phase by the reversal of excess thymidine double-block technique, was kept at 37° and observed as they proceeded into mitosis.

The experimental cells were kept under N_2O for varying periods: 4 hours, 12, 16, and 36 hours. Then they were observed after removal of N_2O at varying intervals: 30 min, 60, and 90 min of incubation at 37°C. When these cells are observed with a light microscope, the chromosomes resemble chromosome arrays produced by colchicine and other spindle poisons. However, when an electron microscope was used, the following differences from colchicine inhibition became apparent.

Colchicine is bound by tubulin; this prevents microtubule assembly. Colchicine effects are irreversible. Nitrous oxide interferes with mitosis in the following ways: 1) bipolar spindles form, with a pair of centrioles

at each pole, but the poles are farther apart (25,000 nm) than the normal distance (10,000 nm); 2) interpolar microtubules form and converge toward the centrioles, and microtubules form, but these chromosomal microtubules do not always orient themselves toward the poles. Attachment to the kinetochore appears normal, but instead of the microtubule being built toward the pole, it often reaches out at right angles, or at random angles, to the pole-pole axis of the spindle. Several microtubules may attach to a single sister kinetochore, and start extending themselves outward at two different angles. Chromosomes seem to be randomly scattered throughout the spindle. Chromosomes did not migrate to the metaphase equatorial plate. Spindle microtubules do not appear to interact to bring about chromosome movement.

If the nitrous oxide treatment lasts for 4 hours, cells recover and proceed through mitosis normally. Pole-to-pole distance shortens to the normal 10,000 nm. Chromosomes are now aligned properly on the equator and a typical bipolar spindle is seen. Metaphase appears normal. Cells approach anaphase in synchrony. Telophase follows without further abnormalities.

However, if N_2O treatment lasts 12 or 16 hours, cells do not recover normally. The number of bipolar cells falls to 55%; tripolar cells comprise 27%; tetrapolar cells comprise 13%. After 36 hours of nitrous

oxide, 42% are tripolar, 40% are bipolar, and 17% are tetrapolar. Even with these multipolar spindles, each pole has a pair of centrioles.

These effects of nitrous oxide do not appear to be due to simple anoxia. When nitrogen gas (N_2) is applied to Hela cells under identical experimental conditions, no increase in mitotic index is seen (Rao, 1968).

Nitrous oxide does not appear to act in the same way as colchicine and other spindle poisons. Unlike colchicine, N_2O does not appear to interfere with microtubule assembly. Spindles form, but chromosomes are not moved about in a normal manner. Chromosome movements appear random and microtubule direction and orientation appear random.

Tubulin makes up at least 25% of the total brain protein. Tubulin is polymerized into microtubules by two other heavier proteins of 290,000 and 310,000 mol wt. GTP is used in the assembly (Borisy et al., 1974). Two moles of GTP are bound per dimer (α,β) tubulin molecule; one of these is tightly bound. Microtubules assemble in a unidirectional way. Kinetochores seem to be the sites for the formation of spindle fibers.

Tubulin, the subunit protein of microtubules, is highly acidic. It can be isolated from the brain, a tissue rich in tubulin, by following a procedure for isolating mitochondria by centrifugation. When the mitochondria

are packed into the pellet, tubulin can be found in the supernatant. This supernatant can be concentrated and warmed, and then the tubulin proteins will assemble themselves into microtubules again. α- and β-tubulin differ in amino acid structure and β-tubulin moves farther under electrophoresis. Both α- and β-tubulin weigh about 52,000. Two larger unknown proteins, of molecular weight 290,000 and 310,000, were found in purified brain tubulin preparations (Borisy, 1974). α- and β-tubulins are associated as heterodimers in protozoa, sea urchins, and chordates.

A microtubule model has been suggested by Bryan (1974) showing the paired α- and β-tubulin proteins winding around the hollow core of the microtubule, like paired strands of light and dark colored wool, or chequered light and dark stitches. A helical turn with a repeat distance of 80 nm and an intrinsic polarity to the microtubule are included in this model. This helix exhibits left-handed chirality in an absolute sense (Snyder and McIntosh, 1976; Erickson, 1974).

Microtubules are, of course, involved in cellular movement in cilia, flagella, and sperm tails. In those structures there seems to be an associated protein, dynein, which is an ATPase. Microtubules transport proteins down an extension of the cell. Thus they are involved in the growth of all eukaryotic cells. Microtubules are involved in root tip growth. Microtubules are associated with

the majority of sensory neurons (Biedler, 1970; Barber, 1974). Exceptions are in vertebrate taste receptors and in the eyes of many invertebrate species. Microtubules in brain tissue bind two moles of ^{3}H-GTP for each dimer of α- and β-tubulin. One molecule of GDP is tightly bound and non-exchangeable. This is then phosphorylated to GTP, using a phosphate from the readily exchangeable GTP. A phosphate is also covalently linked to a serine residue on β-tubulin.

Microtubules are assembled from the α- and β-tubulin proteins which are floating in the cytoplasm. A dynamic equilibrium exists between the free and the assembled tubulin. How is microtubule growth initiated? How is a direction of growth chosen?

Spindle microtubules that are assembled show birefringence (Inoué, 1953). This disappears when colchicine causes the disassembly of microtubules. Birefringence also naturally fluctuates during mitosis since the microtubules are assembling and disassembling as they build the spindle, asters, chromosomal microtubules and then draw the chromosomes to their respective poles. Since cold prevents mitosis from continuing, temperature changes are also reflected in birefringence changes. Any disruption of the ordered array of microtubules is visible as a disruption of birefringence. Microtubule assembly is endothermic and proceeds with a large increase in entropy

(Inoué, 1967). There is a loss of structured water. A large pool of α- and β-tubulin proteins exists constantly and is drawn upon when microtubules are assembled (Raff, 1971). Microtubules seem to be assembled, but not in a spindle array, during interphase; they are disassembled in prophase, and then assembled into the familiar spindle array during metaphase.

Such a dynamic equilibrium for microtubules exists not only in the mitotic spindle formation, but also in neurons, where microtubules constantly form and change. Reassembly is extremely rapid and proceeds regardless of the presence of protein synthesis inhibitors such as actinomycin D. Neurite outgrowth depends on microtubule assembly. Lens cell elongation also depends on the orderly assembly of microtubules. Microtubules play an essential, but mysterious, role in sensory transduction.

Kinetochores seem to act as orienting centers for the newly assembling spindle microtubules. If kinetochores are irradiated with uv light, then microtubules do not assemble, and no birefringence is seen (Inoué, 1964).

Neurons in a developing stage show a very ordered proliferation of microtubules, but there do not seem to be any centers for growth or orientation. Centrioles do not seem to be involved in neuron microtubule growth. Platelets show microtubules oriented around the inner

side of the cell membrane.

Mitotic spindle asters form only in activated oocyte homogenates; if unactivated oocytes are used, or if only the supernatant is used, then the spindle aster will not form. There seems to be some sort of microtubule organizing center in the activated oocyte homogenate which organizes the spindle aster.

Mitosis inducing substances seem to be created during interphase, rising to a critical level just before mitosis. Nitrous oxide at 5.1 atmospheres (80 psi) causes mitotic synchrony in tissue culture cells such as Hela. The mitotic index can be raised to 98%, using first, excess thymidine and second, N_2O. This mitotic block is completely reversible after 20 hours of excess thymidine followed by 10 hours of N_2O (Rao, 1976). Colcemid block is not reversible.

When N_2O was removed from the Hela cells, the mitotic index fell from 98% to 18% within 1½ hours. Cells completed mitosis and entered the G1 growth period. Hela cells ordinarily are mononucleate, with a small (3.75%) group of binucleate cells. N_2O causes this group to increase to 13.5% binucleate cells. This increase is due to N_2O, not to the excess ^3H-thymidine. N_2O also increases the frequency of tripolar and tetrapolar mitotic spindles. Binucleate cells have a shorter G1 growth period than mononucleate cells. Binucleate and trinucleate cells enter the S phase of DNA synthesis with nearly perfect

synchrony. Initiation of DNA synthesis appears to occur independently among the several nuclei, but when one nucleus has begun DNA synthesis, the others follow at once (Rao, 1976). There is some factor which initiates DNA synthesis in S phase cells. These initiating factors are probably proteins.

CHAPTER XI

A THEORETICAL PROPOSAL

Is it possible to construct a hypothesis of the action of nitrous oxide upon neurons which could unite all the diverse effects that have been noted? It seems worth attempting such a project, in spite of the difficulties.

Nitrous oxide affects the microtubules visible in the spindle during mitosis in the following ways:

(1) direction of growth seems random

(2) distance of growth is 2½ times normal

(3) more than one microtubule attaches to the kinetochore

(4) chromosomes are not moved to poles

(5) more than two poles are built on the spindle

(6) cells end up with two nuclei, or three nuclei.

It seems as though nitrous oxide produces a breakdown in communication between the various parts of the mitotic apparatus. We know that the tubulin in the mitotic spindle is the same protein that occurs in microtubules in neurons, using the criteria of molecular weight, amino acid composition, electrophoretic mobility, and immunological specificity. Therefore, it seems logical to infer that

nitrous oxide might affect microtubules in neurons as it does in mitosis.

What might be the result, in neurons, of such an effect upon microtubules? Widespread consequences seem to follow, when we consider the many functions of microtubules. A logical first place to look is at the transport of ATP. We know that ATP is transported by microtubules, since we see this process occurring in sperm tails. The outer ring of 27 microtubules (nine triplets) use GTP as their source of energy, and thus they can transport ATP down the length of the sperm tail. The inner 2 microfilaments of a sperm tail are not tubulin, but dynein, which uses ATP.

While mitochondria make ATP, it still must be transported from the vicinity of the mitochondria over to the place of use, perhaps over to the neuron membrane to be used by the Na^+-K^+ active transport system of proteins, perhaps to the synapse, to the end of the axon or dendrite, or to a neuron membrane protein that makes cyclic AMP. If microtubules cannot "pull" chromosomes out of metaphase toward the poles while under nitrous oxide, perhaps they cannot transport ATP in neurons away from the vicinity of the mitochondria.

What would be the effect of letting the ATP remain close to the mitochondria? The mitochondria would slow down the production of ATP, and hence use less oxygen.

We see this effect in humans or animals who are breathing nitrous oxide and who seem to use less oxygen than normally (Schatte and Bennett, 1973; Schatte et al., 1974; Bradley and Dickson, 1976). But when DiFazio et al. (1969) measured oxygen consumption in rat bone marrow in a Warburg flask, and when Nahrwold and Cohen (1973) measured oxygen consumption in rat liver mitochondria, they found no change in oxygen consumption under N_2O. This puzzling discrepancy makes sense when we realize that these investigators were using bone marrow homogenate or isolated mitochondria. In such preparations, microtubules are thrown away with the supernatant, and the N_2O effect upon microtubules is no longer apparent. The surrounding medium of mitochondria prepared in this way is more watery, contains less protein, and ATP is free to drift away from the mitochondria. Hence, oxygen consumption shows no change in vitro in these preparations.

When Carpenter (1954) used isolated rat sciatic nerve and 90% N_2O at 5 atm, oxygen consumption fell 35%, although no change occurred in action potential. When Carpenter increased 90% N_2O pressure to 13 atm, oxygen consumption fell 65%, and action potential was blocked, but only after 20-30 minutes. We can, using a microtubule loss of function model of N_2O narcosis, make sense of these results also. Oxygen consumption can fall 35%, as ATP are not transported away from mitochondria at the

normal rate, yet still enough ATP reaches the Na^+K^+ active transport system of proteins so that action potential is unimpaired. But when oxygen consumption falls as much as 65%, so little ATP is being transported by microtubules that action potential is blocked.

When C. R. Marshall (1938) experienced unconsciousness with 70% N_2O in 4 minutes, 30 seconds, this might also be due to lack of transport of ATP by microtubules. This effect was produced rapidly, and also was rapidly reversed. Microtubules assemble and disassemble very rapidly, at a rate of 21 tubulin proteins/second.

100% N_2O produces death in less than 45 seconds in a rat. If microtubules are totally disorganized by N_2O, then no ATP would be transported to the appropriate sites, and all action potentials would cease.

The rise in plasma norepinephrine is a complex effect which may also be a result of disrupted microtubules. Olmsted and Borisy (1973) suggest "that microtubules were involved with the mobilization of material for secretion" (p. 510). Such a function, if disturbed by N_2O, could result in sudden release of norepinephrine (N. Ty Smith, 1970), or in release of endorphins or enkephalins (Berkowitz et al., 1976). Bone calcification was interfered with in rat embryos (Fink et al., 1967). Either microtubules did not transport substances properly in the growing vertebrae, or the cell-cell communication system, which

must depend greatly upon the sub-cellular microtubule communication system, was disrupted by N_2O. Orderly growth and development of all cells depends both on the process of mitosis occurring perfectly, and on the protein-transport functions of microtubules continuing in their usual highly coordinated manner.

If nitrous oxide effects upon microtubules interfere with the transport of ATP, this might explain the common subjective feeling of lassitude while breathing N_2O, a "disinclination for work" (Marshall, 1938, p. 424). Ordinarily a release of norepinephrine or other catecholamines results in a feeling of loss of tiredness, an energetic restlessness such as results from an amphetamine pill. Nitrous oxide produces the subjective sensation of a "state of extreme hurry, agitation, and tumult" (Roget, 1799) combined with "a disinclination to motion . . . torpor" (Roget, 1799). Perhaps this effect also results in the characteristic increase in reaction time observed under nitrous oxide (Marshall, 1937; Garfield, 1975; Bradley and Dickson, 1976). "Incoordination of movements becomes noteworthy" (Marshall, 1938). Steinberg (1954) found the motor task "Ball-bearing" to be the task most sensitive to the drug, followed by the motor tasks "Dotting" and "Tapping."

Changes in the immune response, in the bone-marrow production of red blood cells and white blood cells (Lassen,

1956; Eastwood, 1963 and 1964; Green, 1968; M. C. Johnson, 1971) can now be understood as being directly produced by the effects of nitrous oxide upon microtubule function during mitosis. Johnson (1971) supposed that N_2O produced haemopoietic and mitotic changes by means of its ability as a free radical scavenger, but he could find no causal relationships. The causal relationship can now be seen as mediated through the effects of N_2O upon microtubules. Ebert and Hornsey (1958) speculated that N_2O competes with oxygen for access to unidentified but specific sites within the cell, perhaps within the nucleus. The site of action of N_2O now can be seen as upon the microtubules; this does not seem to be a simple competition with oxygen.

Teratogenic effects of nitrous oxide can now be seen as due to the effect of N_2O upon microtubules, both during mitosis and during cell growth, development, and differentiation. An interference with microtubule functioning can explain the death of embryos, and the weight loss in surviving embryos (Fink, 1967). I do not understand why male embryos should be more susceptible to N_2O than females, except to note that this same vulnerability of males extends to a wide variety of other dangers. Fink thought that N_2O affected skeletal growth through direct gene action. N_2O may affect chromosomal proteins. I have found no evidence either way for this hypothesis. But the effect of N_2O upon microtubules is remarkable

enough to affect skeletal growth by itself.

Changes in respiratory frequency (rises) in tidal volume (falls) and in minute volume (rises) which were observed by Bradley and Dickson (1976) may be due to a "second gas" effect of N_2O upon CO_2, causing CO_2 blood levels to remain slightly elevated. Or, as Whitteridge (1944) speculated, N_2O may sensitize pulmonary inflation receptors and deflation receptors, since N_2O causes increased discharge frequencies in both of these groups.

It is difficult to correlate all the effects produced by N_2O upon neuron firing frequencies (Whitteridge and Bulbring, 1944; Mori, 1972; Kitahata, 1973; Sasa, 1967; Hills, 1972). Is this an effect that might follow an effect of N_2O upon microtubules? Microtubules maintain a more negative electrical potential inside the microtubule than exists outside in the cytoplasm, by continually ejecting H^+ (hydrogen) ions while retaining OH^- (hydroxl) ions inside. An action potential, which changes the cytoplasmic potential from -70 mv to +40 mv would certainly produce an effect upon microtubules, which are also exerting energy to maintain a difference in electrical potential. Could such an effect go the other way? That is, could microtubules, by altering their own electrical potential gradient, affect the neuron enough to cause a change in firing rate? If microtubules within the axon do not affect the action potential, still microtubules

within dendrites might affect the summed dendritic post-synaptic potentials, a more finely-tuned and delicate system. If N_2O disturbed the normal functioning of micro-tubules within dendrites, widespread effects could be expected.

Dendrites are the receivers of information in all our neurons. In the rod cells of our retina, dendrites contain 1,000 disks, which each contain 30,000 molecules of rhodopsin protein, each of which holds a molecule of vitamin A in a bent, potentially energizable form. If one photon of light enters the rod cell, and strikes the vitamin A molecule, vitamin A unfolds, straightens out, and thus alters the conformation of its enfolding protein, rhodopsin. This change is amplified and runs along the dendrite of the rod cell. This dendrite was built by a centriole, which exists in the narrow "stem" of the rod cell, between the dendritic part of this neuron and the next volume of neuron, which contains the mitochondria. ATP is certainly transported by the microtubules of this centriole. New proteins, especially rhodopsin, must be transported from the nucleus to the dendrite of the rod cell by the microtubules of the centriole. Vitamin A must be transported there. The whole end of the rod cell, the whole 1,000 disks, is replaced within 3 weeks, a fast turnover of materials.

Suppose N_2O affects microtubules in rod cells.

Could this produce subjective effects such as "suddenly lose sight of all the objects around me, they being apparently obscured by clouds, in which were many luminous points" (Roget, 1799) or "luminous points seemed frequently to pass before my eyes" (Davy, 1799)?

If N_2O affects the functioning of microtubules in dendrites, the sensory processes of all our neurons, then subjective sensations and perceptions of all kinds might follow. Parasthesia, numbness, tingling, "pleasurable thrilling in all the muscles" (Davy, 1799), a feeling of floating or sinking--all these effects from N_2O might follow from its effect upon microtubules in dendrites.

Microtubules assemble themselves at a regular rate, 21 tubulins/second. It takes about 2 minutes to assemble the spindle, a length of 10,000 nm, with about 3,000 microtubules in a spindle (Borisy et al., 1974). Under nitrous oxide this length is extended to 25,000 nm (Brinkley and Rao, 1973). The rate of assembly might also differ under nitrous oxide. Such a change in assembly rate of microtubules may be involved in subjective changes in time estimation. We do not know how humans estimate time, whether they are subconsciously noticing breathing rate (rises under N_2O, Bradley and Dickson, 1976) or heartbeat (no change, Craythorne and Darby, 1965; increased rate, Lundborg, 1966; no change, Ty Smith, 1966; reduced rate, Eisele, 1969). Marshall (1937) said, "time is beginning

to have no meaning for me" (p. 429). Steinberg (1955) found changes in time estimation.

When we consider changes in thought processes brought about by nitrous oxide, we are entering a difficult area of research. Memory and learning are brain functions that have generated a large volume of research, and no great agreement among investigators. Our immune system seems to show a kind of learning and long term memory. The current hypothesis seems to be that all possible antibodies are already coded for in our DNA, and the arrival of an antigen merely accelerates the production of the particular antibody that fits this antigen. Such a model for learning and memory for our immune system seems to be adequate. But if we are to code thoughts and memories in our brains, it does not seem possible that all possible thoughts could be coded in our DNA. Yet if we do not code memories as proteins, how could we code them at all?

Dorothy Pitelka (1974) suggests that the complicated arrangement of microtubules in centrioles, including a "ribbon" that specifies one group among the 9 groups of three microtubules around the centriole, might form such a coding arrangement for learning. This geometic, spatial code would be flexible and adaptable, in contrast to our DNA code, which is linear, digital, and stable. Both kinds of code are necessary for survival.

Tubulin occurs in two forms, α and β. The tubulin

monomers assemble in some regular pattern, perhaps like strands of light and dark wool, or perhaps like a checkerboard pattern, winding around the hollow center of the microtubule. This regular pattern is undisturbed when the neuron or cell is not stimulated. But when a stimulus occurs, any change in the electrical potential of the neuron, or in the composition of the cytoplasm, could result in a change in the regular pattern of assembly. Since 21 tubulins/second are assembled, changes could occur from moment to moment in the process of assembly. Since microtubules maintain an electrical gradient between inside and outside, changes in the cytoplasm could affect the process of assembly and thus alter the pattern.

What might such changes mean in the lifetime of a human being? From the first moments of cell differentiation, responsiveness to outside stimuli, and adaptability to the surrounding environment would be possible. If memories can be coded into a spatial arrangement of proteins, with moment to moment responsiveness, we do not need to delay 20 minutes, in order to build a new protein, in order to code a new memory.

Each one of us feels we are a unique individual, different, not only because of our inherited genetic code, but also because of our own unique environment, our own experiences since earliest childhood. If we suppose our memories to be coded in our centrioles and microtubules,

our uniqueness is comprehensible, since even identical twins would develop differently coded centrioles.

Nitrous oxide produces "a loss of all sense of self . . . a feeling of non-existence . . . an awakening from death to life" (Marshall, 1938). Subjects feel far away, detached. Amnesia sometimes occurs. If our thoughts and memories are indeed encoded by centrioles and microtubules, it is not surprising that changes produced by nitrous oxide upon these structures should produce such remarkable subjective effects.

Nitrous oxide does not halt the formation of microtubules; it does alter their orientation. This disruption might account for those experiences typical of psychedelic states: the experience of new meaning, the juxtaposition of thoughts and feelings in new and unexpected ways which produce mirth and insight, the rapid and kaleidoscopic flow of ideas and images. All of these effects might follow from the effects of N_2O upon neuronal microtubules.

Past theories about the mechanism of action of nitrous oxide have considered its lipid solubility, its ability to form a hydrate microcrystal, its polarizability, surface film affinity, mole volume, and dipole moment. It has been seen as affecting cation permeability, and as releasing endogenous opiates. It has been seen as acting at the synapse, or within the lipid membrane. Whenever we approach one of these theories, we must keep in mind

substances which do have the property or action being considered, but which do not produce the narcosis or analgesia. For example, camphor has a high lipid solubility coefficient, yet has no anesthetic properties. Nicotine selectively blocks synaptic transmission more than any narcotic or analgesic, yet nicotine does not act as an anesthetic. The Na^+-K^+ active transport protein assembly is selectively blocked by the cardiac glycosides, ouabain, digitoxigenin, or scillaridin, more than by any narcotic or analgesic, yet these drugs do not act as anesthetics.

Because of my dissatisfaction with such past theories, I kept on searching for an effect of nitrous oxide that I felt might encompass not only a particular physiological effect, but many physiological effects, even behavioral effects, and even experiential effects. Whether the effects of nitrous oxide upon microtubules will actually prove to become an accepted theory of action of nitrous oxide is for others to determine. For myself, the interest of the search, the wonder of discovery, the miraculous awareness of proteins that live within our neurons--these brought me ample return for the work involved.

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APPENDIX I

SUBJECTIVE SENSATIONS UNDER NITROUS OXIDE

PETER MARK ROGET

"The first effect was that of making me vertiginous, and producing a tingling sensation in my hands and feet; I seemed to lose the sense of my own weight, and I imagined I was sinking into the ground. I then felt a drowsiness gradually steal upon me, and a disinclination to motion; even the actions of inspiring and expiring were not performed without effort; and it also required some attention of mind to keep my nostrils closed with my fingers. I was gradually roused from this torpor by a kind of delirium; which came on so rapidly that the air-bag dropt from my hands and I suddenly lost sight of all the objects around me, they being apparently obscured by clouds, in which were many luminous points, similar to what is often experienced on rising suddenly and stretching out the arms, after sitting long in one position. I felt myself totally incapable of speaking, and for some time lost all consciousness of where I was or who was near me. My whole frame felt as if violently agitated; I thought I panted violently; my heart seemed to palpitate and every artery to throb with violence; I felt a singing in my ears: all the vital motions seemed to be irresistibly hurried on as if their equilibrium had been destroyed

and everything was running headlong into confusion. My ideas succeeded one another with extreme rapidity, thoughts rushed like a torrent through my mind, as if their velocity had been suddenly accelerated by the bursting of a barrier which had before retained them in their natural and equable course. This state of extreme hurry, agitation, and tumult, was but transient. Every unnatural sensation gradually subsided.

"I am sensible that the account I have been able to give of my feelings is very imperfect. For however calculated their violence and novelty were to leave a lasting impression on the memory, these circumstances were for that very reason unfavorable to accuracy of comparison with sensations already familiar.

"The nature of the sensations themselves, which bore greater resemblance to a half-delirious dream than to any distinct state of mind capable of being accurately remembered, contributes very much to increase the difficulty."

HUMPHREY DAVY

". . . the pleasurable delirium . . . a sensation analogous to gentle pressure on all the muscles, attended by a highly pleasurable thrilling . . . The objects around me became dazzling and my hearing more acute . . . I often thought that it produced a feeling somewhat analogous

to taste, in its application to my lungs . . . When ten quarts had been breathed for near 4 minutes, an exhilaration and a sense of slight intoxication lasted for 2 or 3 hours . . . At other times, I had sublime emotions connected with highly vivid ideas . . . Previous to sleep, my mind was long occupied with visible imagery . . . I imagined that I had increased sensibility of touch . . . Headache was wholly removed by two doses of the gas . . . I was unconscious of headache after the third inspiration . . . I resolved to breathe the gas (N_2O) for such a time and in such quantities, as to produce excitement equal in duration and superior in intensity, to that occasioned by high intoxication from opium or alcohol . . . I had now a great disposition to laugh, luminous points seemed frequently to pass before my eyes, my hearing was certainly more acute and I felt a pleasant lightness and power of exertion in my muscles . . . I felt a sense of tangible extension highly pleasurable in every limb; my visible impressions were dazzling and apparently magnified; I heard distinctly every sound in the room and was perfectly aware of my situation. By degrees as the pleasurable sensations increased, I lost all connection with external things; trains of vivid visible images rapidly passed through my mind, and were connected with words in such a manner as to produce perceptions perfectly novel. I existed in a world of newly connected and newly modified

ideas. I theorized; I imagined that I made discoveries . . . I continued in the pleasurable trance longer than before; the exhilaration continued nearly two hours . . . I have often felt very great pleasure when breathing it alone, in darkness and silence, occupied only by ideal existence . . . Whenever I have breathed the gas after excitement from moral or physical causes, the delight has been often intense and sublime.

"From the nature of the language of feeling the preceding detail contains many imperfections. I have endeavored to give as accurate an account as possible of the strange effects of nitrous oxide, by making use of terms standing for the most familiar common feelings . . . I have sometimes experienced from nitrous oxide, sensations similar to no others, and they have consequently been indescribable." (pp. 458-491)

BENJAMIN PAUL BLOOD

"The anesthetic revelation is the initiation of man into the Immemorial Mystery of the Open Secret of Being, revealed as the inevitable Vortex of Continuity . . . My grey gull lifts her wings against the night-fall, and takes the dim leagues with a fearless eye . . . And now, after twenty-seven years of this experience [breathing N_2O], the wing is greyer, but the eye is fearless still, while I renew and double emphasize the declaration. I

know--as having known--the meaning of existence: the same center of the universe--at once the wonder and assurance of the soul--for which the speech of reason has as yet no name but the Anaesthetic Revelation."

WILLIAM JAMES

"Some observations of the effects of nitrous oxide gas intoxication . . . With me, as with every other person of whom I have heard, the keynote of the experience is the tremendously exciting sense of an intense metaphysical illumination. Truth lies open to the view in depth beneath depth of almost blinding evidence. The mind sees all the logical relations of being with an apparent subtlety and instantaneity to which its normal consciousness offers no parallel; only as sobriety returns, the feeling of insight fades, and one is left staring vacantly at a few disjointed words and phrases, as one stares at a cadaverous-looking snow-peak from which the sunset glow has just fled, or at the black cinder left by an extinguished brand.

"The immense emotional sense of reconciliation which characterizes the "maudlin" stage of alcoholic drunkenness--a stage which seems silly to lookers-on, but the subjective rapture of which probably constitutes a chief part of the temptation to the vice--is well known. The centre and periphery of things seem to come together.

The ego and its objects, the meum and the tuum, are one. Now this, only a thousandfold enhanced, was the effect upon me of the gas; and its first result was to make peal through me with unutterable power the conviction that Hegelism was true after all, and that the deepest convictions of my intellect hitherto were wrong. Whatever idea of representation occurred to the mind was seized by the same logical forceps, and served to illustrate the same truth; and that truth was that every opposition, among whatsoever things, vanishes in a higher unity in which it is based; that all contradictions, so-called, are but differences; that all differences are of degree; that all degrees are of a common kind; that unbroken continuity is of the essence of being; and that we are literally in the midst of an infinite, to perceive the existence of which is the utmost we can attain . . . it is impossible to convey an idea of the torrential character of the identification of opposites as it streams through the mind in this experience. I have sheet after sheet of phrases dictated or written during the intoxication . . . which at the moment of transcribing were fused in the fire of infinite rationality . . . Good and evil, life and death, I and thous, matter and form, shiver of ecstasy and shudder of horror, fate and reason, and fifty other contrasts figure in these pages. The thought of mutual implication of the parts . . . produced

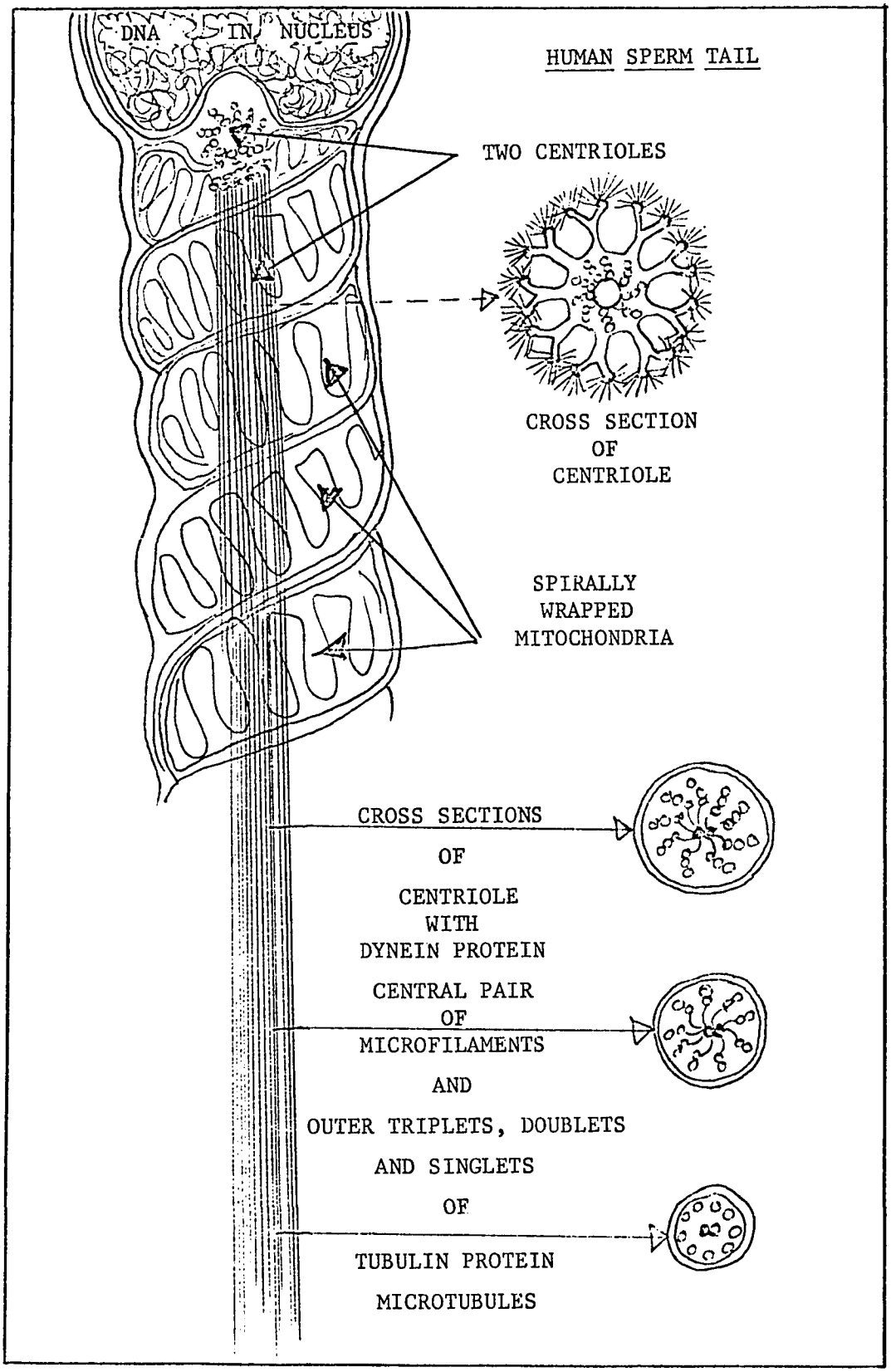
a perfect delirium of theoretic rapture.

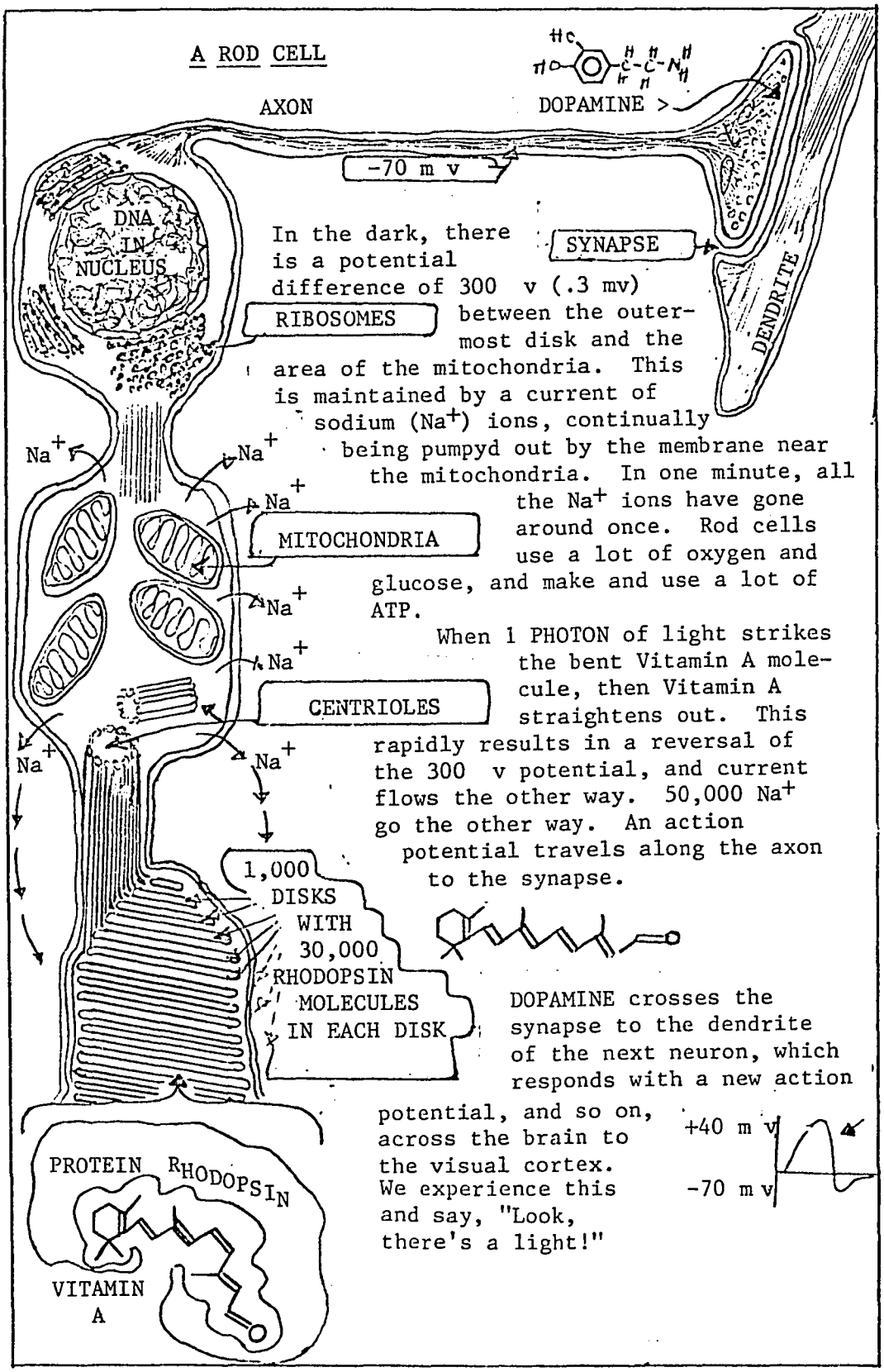
"What is the principle of unity in all this monotonous rain of instances? . . . Nothing but the abstract genus of which the conflicting terms were opposite species. The flood of ontologic emotion was Hegelian through and through . . . At the same time the rapture of beholding a process that was infinite, changed (as the nature of the infinitude was realized by the mind) into the sense of a dreadful and ineluctable fate, with whose magnitude every finite effort is incommensurable and in the light of which whatever happens is indifferent. This instantaneous revulsion of mood from rapture to horror is, perhaps, the strongest emotion I have ever experienced . . . A pessimistic fatalism, depth within depth of impotence and indifference . . . whichever you choose it is all one.

"Something 'fades,' 'escapes'; and the feeling of insight is changed into an intense one of bewilderment, puzzle, confusion, astonishment. I know no more singular sensation than this intense bewilderment." (pp. 186-208)

APPENDIX II

FIGURES AND CHEMICAL FORMULAE

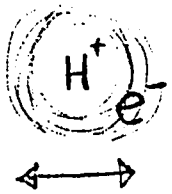

DNA IN NUCLEUS
HUMAN SPERM TAIL
TWO CENTRIOLES
CROSS SECTION OF CENTRIOLE
SPIRALLY WRAPPED MITOCHONDRIA
CROSS SECTIONS OF CENTRIOLE WITH DYNEIN PROTEIN CENTRAL PAIR OF MICROFILAMENTS AND OUTER TRIPLETS, DOUBLETS AND SINGLETS OF TUBULIN PROTEIN MICROTUBULES



In the dark, there is a potential difference of 300 v (.3 mv) between the outermost disk and the area of the mitochondria. This is maintained by a current of sodium (Na^+) ions, continually being pumpyd out by the membrane near the mitochondria. In one minute, all the Na^+ ions have gone around once. Rod cells use a lot of oxygen and glucose, and make and use a lot of ATP.

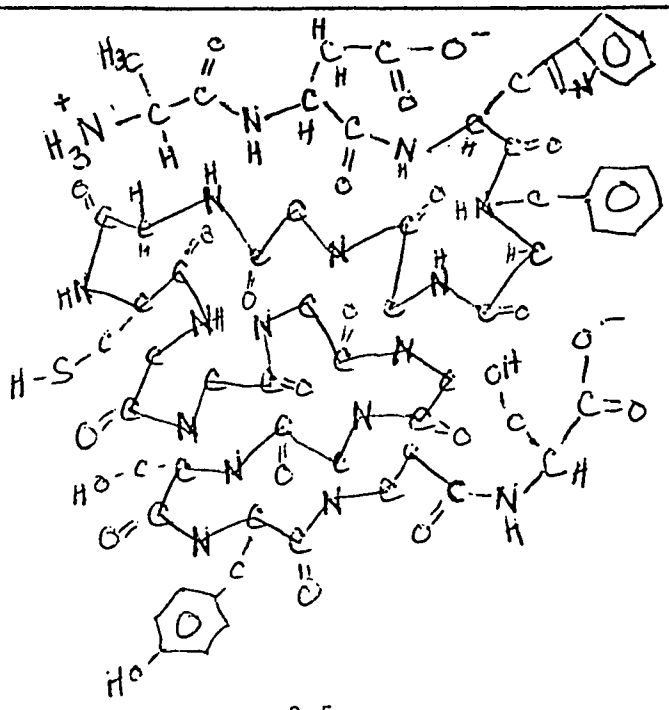
When 1 PHOTON of light strikes the bent Vitamin A molecule, then Vitamin A straightens out. This rapidly results in a reversal of the 300 v potential, and current flows the other way. 50,000 Na^+ go the other way. An action potential travels along the axon to the synapse.

DOPAMINE crosses the synapse to the dendrite of the next neuron, which responds with a new action potential, and so on, across the brain to the visual cortex. We experience this and say, "Look, there's a light!"



0.1 n m

one hydrogen atom

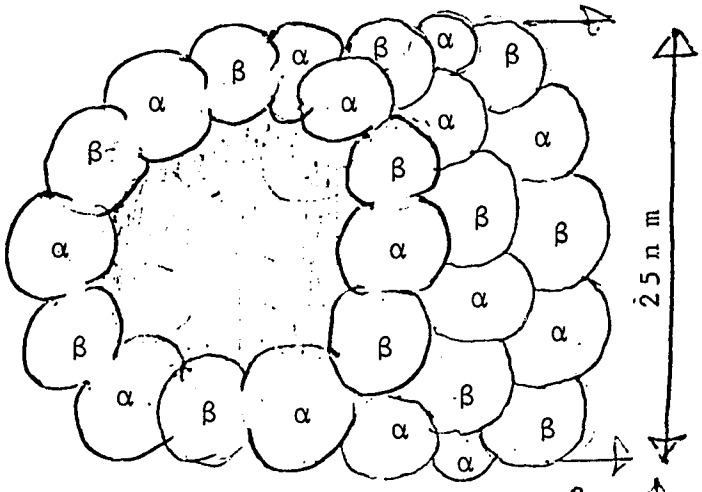


3.5 n m

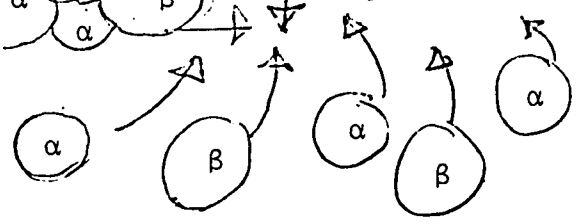
α-tubulin or β-tubulin

≈ 55,000 mol. wt

≈ 458 amino acids



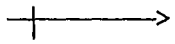
one microtubule, is built at a rate of 21 tubulins per second



microtubules are constantly assembling and disassembling

$\delta -0.33 \quad \delta +1.08 \quad \delta -0.75$ Nitrous Oxide is a resonance hybrid.

$N \equiv N \equiv O$



0.167 = dipole moment
debyes

polarizability = 3.2 cc x 10^{24}

Van der Waals =

(a) 3.78 $liters^2$ (atm)/$moles^2$

attractive force between molecules

(b) 0.0442 liters/mole

finite volume and incompressibility of molescules

Vapor density = 1.5; compared to air = 1

Specific gravity = 1.977; compared to water = 1

Molecular weight = 44.02; compared to CO_2 = 44.01

Partition coefficients

blood/gas = 0.47/1; compared to N ≡ N which is 0.0138

lipid/water = 3.2/1; compared to N ≡ N at 3.5/1

Lipid solubility

ml of gas/liter of lipid = 1,400 ml/liter; compared to N ≡ N at 67 ml/liter

compared to: Ether at 65,000 ml/liter Argon at 140 ml/liter

Cyclopropane at 11,200 ml/liter Krypton at 430 ml/liter

Halothane at 224,000 ml/liter Xenon at 1,700 ml/liter

Methoxyflurane at 825,000 ml/liter Ethylene at 1,300 ml/liter

ANESTHETICS

Methoxyflurane
Enflurane
Halothane
Cyclopropane
Fluroxene
Chloroform
Ether
Trilene

INERT GASES

Hydrogen	H-H
Helium	He
Nitrogen	N ≡ N
Neon	Ne
Argon	Ar
Krypton	Kr
Xenon	Xe

ANALGESICS

Fentanyl
Heroin
Morphine
Codeine Cocaine
Percodan Aspirin
Methadone Cocaine
Demerol
Xylocaine

AMNESIACS Scopolamine

N ≡≡ N ≡ O

SPINDLE POISONS

Colchicine
Vinblastine
Podophyllotoxin
Griseofulvin

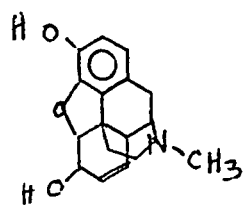
Veronal	Alcohol	Valium
Phenobarbital	Ether	Librium
Nembutal	Opium	Equanil
Seconal	Cocaine	Quaalude
Thiopental	Marijuana	Dalmane
	Psilocybin	Tofranil
	L.S.D.	Thorazine
	Mescaline	

HYPNOTICS:

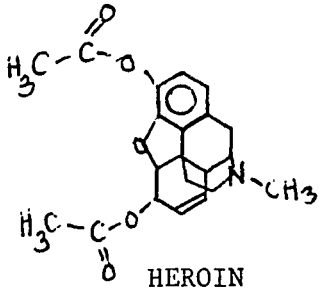
CALM
ASLEEP
UNCONSCIOUS

EUPHORICS
PSYCHEDELICS

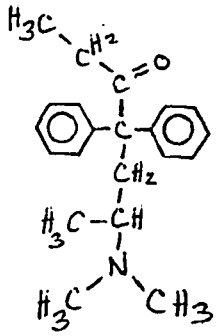
ANTI-ANXIETY DRUGS



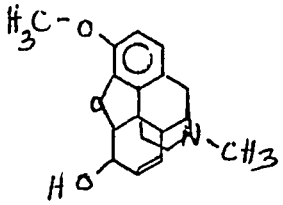
MORPHINE



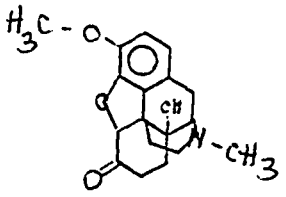
HEROIN



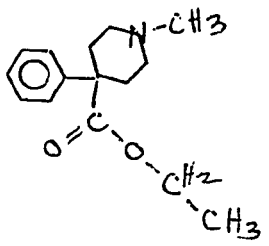
METHADONE



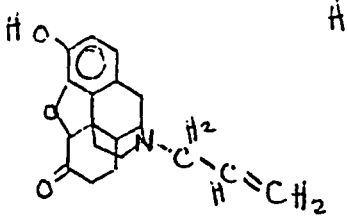
CODEINE



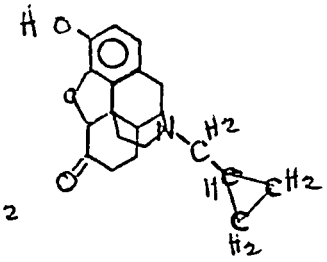
PERCODAN



DEMEROL

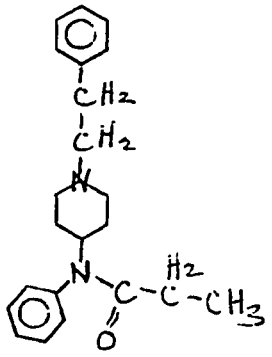


NALOXONE

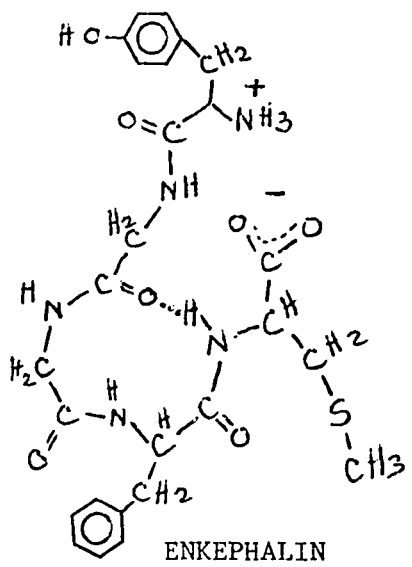


NALTREXONE

(MORPHINE ANTAGONISTS)



FENTANYL

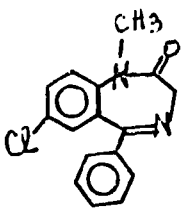


ENKEPHALIN

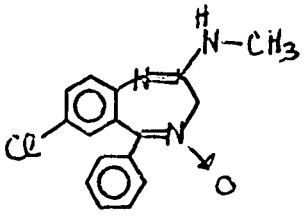
OPIATES

AND

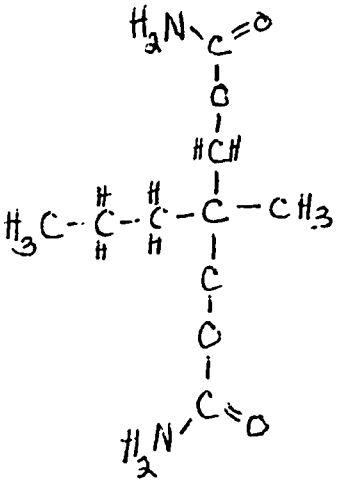
ANALGESICS



VALIUM

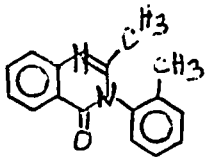


LIBRIUM

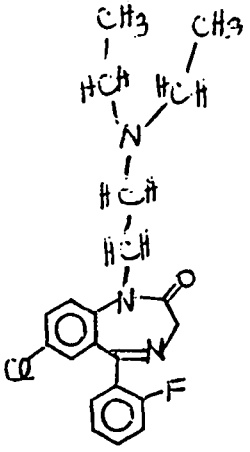


EQUANIL

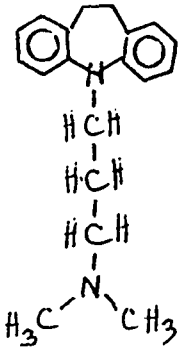
ANTI-ANXIETY DRUGS



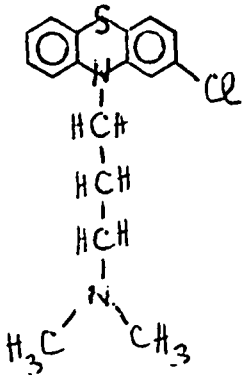
QUAALUDE



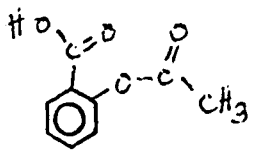
DALMANE



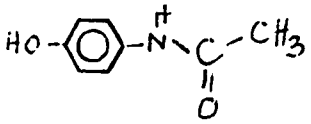
TOFRANIL



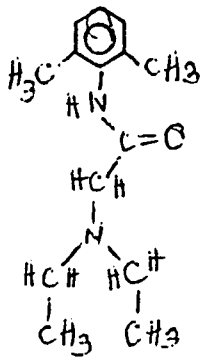
THORAZINE



ASPIRIN

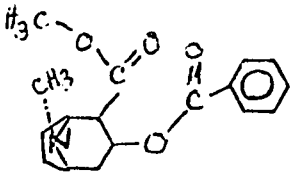


TYLENOL



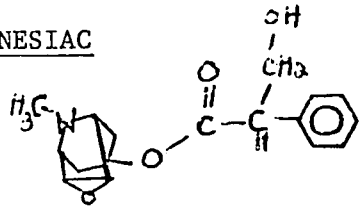
XYLOCAINE

ANALGESICS

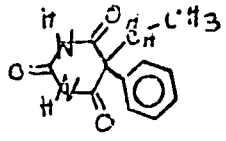


COCAINE

AMNESIAC

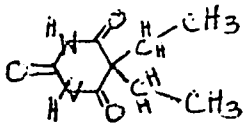


SCOPOLAMINE



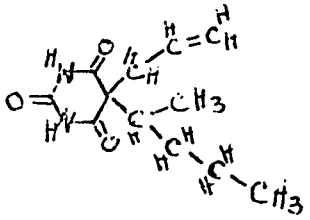
PHENOBARBITAL

(LIPID SOL. = 3)



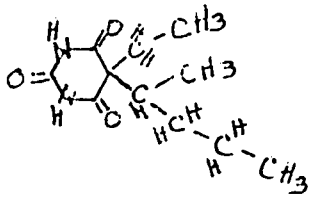
VERONAL

(LIPID SOL. = 1)



SECONAL

(LIPID SOL. = 52)



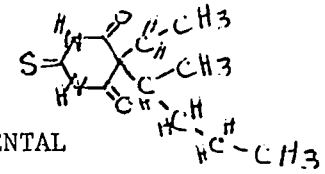
NEMBUTAL

(LIPID SOL. = 39)

HYPNOTICS: CALM,

ASLEEP,

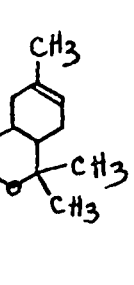
UNCONSCIOUS



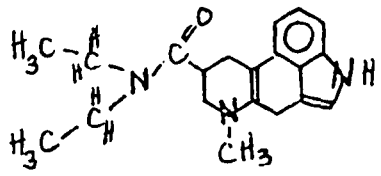
THIOPENTAL

(LIPID SOL. = 580)

BARBITURATES

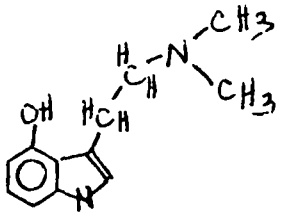


NITROUS OXIDE

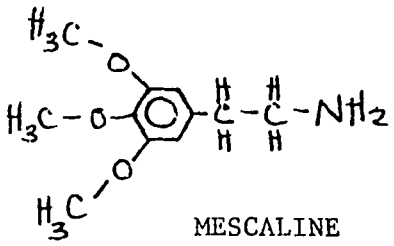


LYSERGIC ACID DIETHYLAMIDE (L.S.D.)

Δ^9 TETRA HYDRO CANNABINOL (MARIJUANA RESIN)



PSILOCIN (FROM PSILOCYBIN)

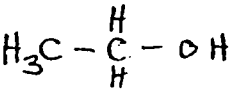


MESCALINE (FROM PEYOTE)

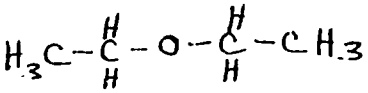
EUPHORICS

PSYCHEDELICS

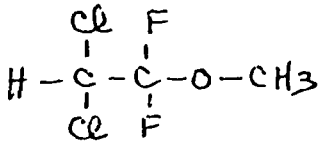
ANESTHETICS



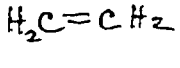
ETHYL ALCOHOL



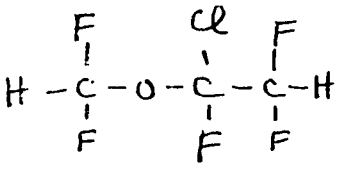
DIETHYL ETHER



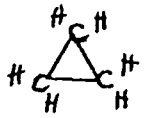
METHOXYFLURANE



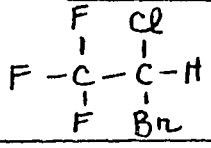
ETHYLENE



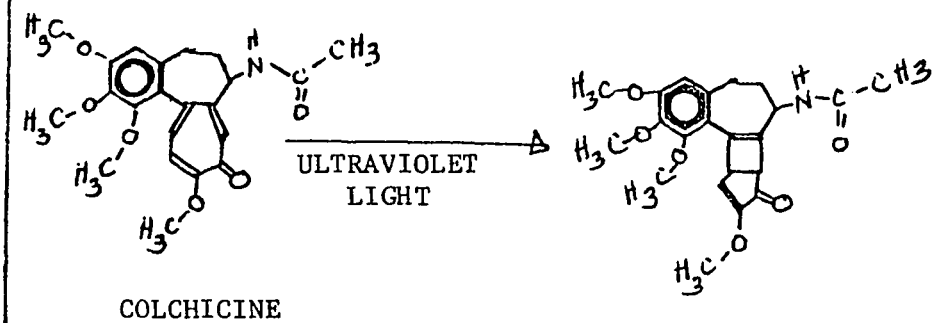
ENFLURANE



CYCLOPROPANE



HALOTHANE

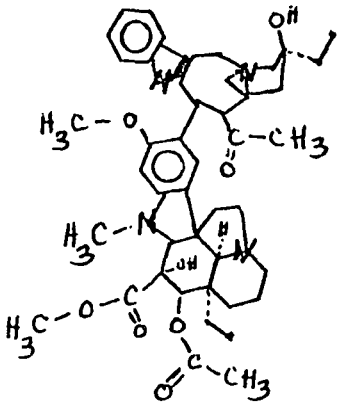


COLCHICINE

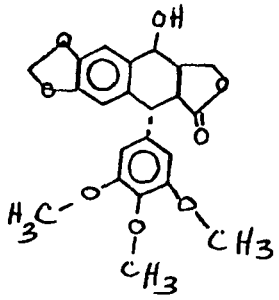
(binds tubulin)

LUMICOLCHICINE

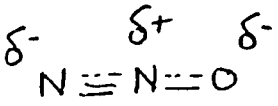
(does not bind tubulin)



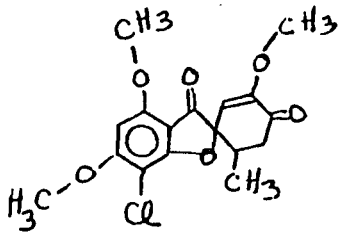
VINBLASTINE



PODOPHYLLOTOXIN



NITROUS OXIDE



GRISEOFULVIN

SPINDLE POISONS