A STUDY TO ASCERTAIN WHETHER SCIENTIFIC DISCRIMINATION WAS USED
BY PROFESSIONAL NURSES IN GIVING OXYGEN THERAPY
FROM A P.R.N. ORDER

by

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A Study to Ascertain Whether Scientific Discrimination was Used by Professional Nurses in Giving Oxygen Therapy from a P.R.N. Order

Thesis directed by Associate Professor Edith Olson and Associate Professor Patricia Vander Leest

The problem of the study was to ascertain whether or not a selected group of professional nurses used scientific discrimination in the process of identifying overt signs and symptoms of anoxia upon which they then made the judgment to administer oxygen from a p.r.n. order. The purposes of the study were: (1) to identify the signs and symptoms of anoxia which are based on scientific, physiological principles; (2) to ascertain what overt signs and symptoms professional nurses used in making a decision to administer oxygen from a p.r.n. order; (3) to determine if certain systemic categories of signs and symptoms were recognized more frequently than others; and (4) to ascertain whether or not professional nurses used a scientific rationale in making a decision to give oxygen therapy.

A review of scientific and nursing literature was made in order to gain an understanding of the physiology of anoxia, its signs and symptoms, and the indications for oxygen therapy. The review of the nursing literature revealed that the description of the physiology of anoxia and its signs and symptoms was far from inclusive. Instead, the emphasis was on the methods and procedures...
for the administration of oxygen therapy. Yet when a medical order for oxygen therapy on a p.r.n. basis has been written, professional nurses must assume the responsibility for a decision as to whether or not oxygen therapy is indicated.

The population of the study consisted of 100 professional nurses currently employed on the medical, surgical, pediatric, and intensive care units of a general hospital. This hospital was connected with a University Medical Center in the Rocky Mountain area. The descriptive survey method with the check list as the data gathering technique was used in this study. Categorization and tabulation was the method of analysis.

On the basis of the data obtained in this study the following major conclusions were drawn: (1) since only two well-known and advanced overt signs and symptoms (dyspnea and cyanosis) were identified with consistency by the respondents, this group of professional nurses was not aware of the many overt signs and symptoms of anoxia in which oxygen therapy might be of benefit; and (2) with the possible exception of those overt signs and symptoms of anoxia related to the circulatory system, their use of scientific discrimination was not evident.

This abstract of about 250 words is approved as to form and content. I recommend its publication.

Signed

Edith V. Olson

I. Vander Leest
Instructors in charge of thesis
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The Problem

Statement of the problem.—The problem was to ascertain whether or not a selected group of professional nurses used scientific discrimination in the process of identifying overt signs and symptoms of anoxia upon which they then made the judgment to administer oxygen from a p.r.n. order.
Purposes of the study.—The purposes of this study were:

(1) to identify the signs and symptoms of anoxia which are based on scientific, physiological principles; (2) to ascertain what overt signs and symptoms professional nurses used in making a decision to administer oxygen from a p.r.n. order; (3) to determine if certain systemic categories of signs and symptoms were recognized more frequently than others, and (4) to ascertain whether or not professional nurses used a scientific discrimination in making a decision to give oxygen therapy.

Justification for the study.—The decisions of nurses as to whether or not to administer oxygen therapy need to be based on a knowledge of the physiology of anoxia and the signs and symptoms which it may manifest. While other scientific literature is replete with discussions of anoxia, nursing literature is very limited in its explanation of the physiology of anoxia, and there are no complete lists of the signs and symptoms which can arise because of anoxic conditions. The major portion of the nursing literature regarding oxygen therapy is devoted to methods of administration and safety precautions. Yet, when the physician has left an order for oxygen to be given on a p.r.n. basis, professional nurses are expected to assume the responsibility for making a decision as to whether or not oxygen therapy is indicated.
While one would expect to find a thorough discussion of the methods of administration in the fundamental or basic textbooks for nurses, one would also expect to find a rather complete list of the signs and symptoms which could be indicative of anoxia. This information would aid nurses in making the judgment as to when oxygen therapy was indicated. Yet, it was found that the preparational background provided for the nurse in this area was very sparse. The texts in medical-surgical nursing vary in their statements of the signs and symptoms of anoxia. Some list three or four, and others include eight to ten. However, as many as thirty-two signs and symptoms of anoxia which would indicate the use of oxygen therapy were found in the scientific literature. Of these thirty-two signs and symptoms, twenty-three were definitely overt.

The disparity in the amount of signs, symptoms, and physiology in the nursing literature as compared to that in the scientific literature justified this type of study. It was important to know whether professional nurses relied only on a few obvious and perhaps


advanced signs and symptoms in making their judgment regarding the necessity for oxygen therapy when a p.r.n. order has been written by the physician, or whether through experience or some other means they had accumulated a knowledge of the signs and symptoms of anoxia which indicate the need for oxygen therapy.

Scope and limitations.—The study was conducted in a University Medical Center Hospital which was located in a large metropolitan area of the Rocky Mountains. It had a bed capacity of 437. Professional nurses involved in the study were those that held staff positions on the general medical, surgical, and pediatric units and the intensive care units. These nurses were employed on all three shifts of duty. There was no inhalation therapy department in this hospital; thus professional nurses were responsible for the administration of oxygen therapy. For the purposes of this study, references made to anoxic conditions included those patients with acute anoxia and eliminated those with chronic, long-standing anoxia. Only the overt signs and symptoms of anoxia were used as the basis for nurses to indicate their decisions in giving oxygen therapy from a p.r.n. order. Since the study was conducted over a two-week period only and was limited to one hospital in the Rocky Mountain area, the findings of the study cannot be generalized to other hospitals or areas.

Methodology.—The method chosen for the study was the normative or descriptive survey because it is a way of obtaining facts about a current situation. This method provided a way of approaching the
problem to ascertain the signs, symptoms, and scientific discrimination professional nurses were currently using as the means of identifying an anoxic patient and upon which they then made the judgment to administer oxygen from a p.r.n. order.

The check list was used as the technique for data collection. Data from the check list were classified into categories and transferred to a work sheet so that tabulation could be done. This ordered the data in a meaningful way in preparation for analysis.

Definition of Terms

For the purposes of this study, the following definitions of terms were used.

Nurse. An individual who has completed a course in nursing in a state approved school and who is currently licensed by the state to practice professional nursing.

Judgment. A conclusion arrived at after consideration of the presenting signs and symptoms which thereby initiates appropriate action.

P.R.N. An order which may be put into effect as circumstances may require.

Signs and symptoms. Evidence of an abnormal nature in the body which the nurse may perceive objectively or subjectively.

Overt. A sign or symptom which is apparent to the nurse either by direct observation or by a direct statement from the patient.
Anoxia. The term is used interchangeably with hypoxia to imply any oxygen deficiency in the body which is great enough to give rise to overt signs and symptoms and thus warrant the use of oxygen therapy.

Scientific discrimination. A distinction or discernment made on the basis of physiological principles.

Organization of the Remainder of the Thesis

Chapter II contains a review of literature concerning the physiology of respiration, the physiology of anoxia with its manifested signs and symptoms, the conditions which can produce anoxia, the indications for oxygen therapy, and the related nursing literature in this area. Chapter III explains the methodology. The descriptive survey is reviewed and the technique for collecting the data is described and discussed. Chapter IV includes tabulations, analysis, and interpretation of the data obtained. Summary, conclusions, and recommendations for further study are found in Chapter V.
CHAPTER I

REVIEW OF THE LITERATURE

In order to ascertain the causes of anoxia, its signs, symptoms, and treatment, and the established nursing practices in regard to anoxia, a survey was made of nursing periodicals and textbooks, medical periodicals, research literature, and physiology textbooks. Since medical knowledge is rapidly increasing and changing, the concentration of review was made of literature which had been published almost exclusively since 1960.

The Physiology of Respiration

Respiration is the exchange of gases between the organism and its environment. The physiology of respiration involves several distinct processes: the moving of air into the lungs; the transfer of gases from the air in the lungs to the blood and then to the cells; and the cellular respiration where oxygen is utilized for the production of energy. Simultaneously, there is a reverse order of the processes in which the carbon dioxide produced as a waste product...
of cellular respiration is carried by the blood and eliminated through
the lungs. The lungs are the primary site of exchange for oxygen intake
and carbon dioxide elimination in respiration. However, the exchange
also involves the respiratory tract and the respiratory muscles.

Included in the respiratory tract are the nose, pharynx, trachea,
bronchi and alveoli of the lungs. The respiratory muscles include
the diaphragm and the abdominal and intercostal muscles. The moving
of air into and out of the respiratory tract and the exchange of
gases are due in part to the difference between the negative pressure
of the pleural cavity and the pressure of the atmosphere. This
exchange is in accordance with Dalton's law and Henry's law.

Dalton's law states that in a mixture of gases, each gas
exerts a pressure as if it were the only gas present; so the
pressure of each gas is proportional to its percentage in the
mixture...Henry's law states that when the temperature remains
constant, the quantity of a gas which will go into a solution
(dissolve in a liquid) is proportional to the partial pressure
of that gas.

Or stated more simply, a gas will flow from a high pressure area
to a low pressure area. The other supplement to this exchange is
the result of the contraction and the relaxation of the respiratory
muscles. These constitute movements associated with breathing and
further accelerate pressure changes.

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3 W. W. Tuttle and Byron A. Schottelius, Textbook of Physiology

The rhythm, rate, and depth of breathing are determined by the respiratory center in the medulla oblongata. The respiratory center is stimulated by the chemical condition of the blood, namely, tension of the carbon dioxide (normal 40 mm. Hg.) and of the oxygen, and the concentration of hydrogen ions. The normal acid-base balance of the blood has a ratio value of pH 7.4. The discharging of more or less carbon dioxide from the blood by the respiratory system is an important factor in maintaining the proper acid-base balance of the body.\(^5\) Impulses from the respiratory center are passed to the muscles of respiration by the phrenic and intercostal nerves. There is stimulation of other nerve pathways leading to the larynx, the muscles of the mouth, the external nares, and the smooth muscles of the bronchioles. Thus several complex conditions are involved in the physiology of respiration.

The Physiology of Anoxia

Anoxia can occur as a disruption or impairment in any of the processes which are involved in normal respiration. Guyton gives a classification of anoxia according to physiological principles, as follows:

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1. Atmospheric anoxia
2. Hypoventilation anoxia
   a. Paralysis of the respiratory muscles
   b. Increased airway resistance
   c. Decreased pulmonary compliance
   d. Increased pulmonary tissue resistance
3. Diffusion anoxia
   a. Reduced active pulmonary membrane area
   b. Thickened pulmonary membrane
4. Abnormal ventilation - perfusion ratio anoxia
   a. Abnormal alveolar ventilation-perfusion ratio
   b. Right-to-left shunts in the lungs or heart
5. Hypohemoglobinemic anoxia
   a. Hemoglobin deficiency (usually caused by anemia)
   b. Carbon monoxide poisoning
   c. Methemoglobinemia
   d. Other abnormalities of hemoglobin
6. Ischemic anoxia
   a. Generalized decrease in cardiac output
   b. Localized tissue ischemia
7. Tissue utilization anoxia
   a. Poisoning of tissue oxidative enzymes
   b. Tissue edema causing poor oxygen diffusion
   c. Excessive tissue demand for oxygen

An understanding of the principles in this classification is necessary for knowing whether or not oxygen therapy can be beneficial. According to Guyton, oxygen therapy can completely correct the depressed oxygen level in atmospheric anoxia. Atmospheric anoxia frequently occurs at high altitudes. Deleterious effects are more pronounced at elevations of 8,000-14,000 feet above sea level than at lower elevations. Altitudes between 5,000 and 8,000 feet may be detrimental to a patient's condition when this height is combined with any of the diseases or conditions which may cause anoxia.

According to Brown, early symptoms of hypoxia may occur at 5,000 feet. He stated:

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The retina which is an outcropping of the brain, is more demanding of adequate oxygen than any other part of the body. For this reason, the first evidence of hypoxia occurs at 5,000 feet in the form of diminished night vision. We should constantly be conscious of this night vision problem, and make allowances for it.

The reason people exposed to high altitudes develop symptoms of acute hypoxia is that there is a decrease of the partial pressure of oxygen in atmospheric air.

In atmospheric anoxia, as in any type of anoxia, the earliest effects are upon the nervous system. Neurological manifestations vary from impairment of the highest cerebral functions in mild anoxia to convulsions and irreversible cerebral damage in severe anoxia. Other symptoms of central nervous system hypoxia are an initial feeling of well-being succeeded by any of the following: tachycardia, hyperventilation, pallor, precordial pain, severe joint discomfort, vomiting, loss of memory, loss of judgment, frequent yawning or sighing, lack of fine muscle coordination, restlessness, mental depression and headache. The headache which occurs in anoxia from high altitude is caused by cerebral vascular distention. "This intense throbbing headache is a sensation of fullness of the head, hot flashes of the face and deep cyanosis."
A person with hypoventilation anoxia can move five times as much oxygen into the alveoli with each breath by breathing 100 percent oxygen as compared to breathing normal air. Therefore, here again oxygen therapy can be extremely beneficial. In diffusion anoxia, essentially the same result occurs as in hypoventilation anoxia, for oxygen therapy can increase the oxygen partial pressure in the lungs from about 100 mm. Hg. to as high as 600 mm. Hg. This causes a greatly increased diffusion gradient between the alveoli and blood.

Some of the conditions which may cause hypoventilation anoxia, diffusion anoxia, or abnormal ventilation are pneumothorax, lung cysts, pulmonary congestion and edema, lobar pneumonia, bronchitis, tuberculosis, pulmonary fibrosis, silicosis, cancer, asthma, emphysema, and foreign objects. The frequent signs and symptoms associated with these conditions are cyanosis, dyspnea, and tachycardia. Oxygen therapy may be of help in anoxia which is caused by asthma, if the asthma is associated with heart disease. Caution must be maintained because there may be an equal difficulty with oxygen uptake and carbon dioxide excretion. Emphysema is another chronic lung condition where caution must be used in administering oxygen therapy. Simonson and McGavack felt that oxygen was indicated in patients with emphysema during the course of acute respiratory failure or during an acute

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exacerbation of the chronic infection already present. They pointed out the dangers of hypoventilation and further accumulation of carbon dioxide until carbon dioxide narcosis might ensue. They advised avoiding this complication by starting oxygen at a low rate of flow — 1 liter/minute — and gradually raising by 1 liter/minute/day until a concentration of 35 to 40 per cent was obtained in the inhaled air.

The presence of anoxic conditions may be indicated by the carbonic acid level of the blood. Thus laboratory reports can supply additional information regarding the necessity for oxygen therapy. According to Guyton, the blood carbonic acid level of 40 mm. Hg. is usually reduced in anoxia. However, in hypoventilation the carbonic acid level rises. An extreme rise could be indicative of carbon dioxide narcosis, and oxygen would be contra-indicated. With a rise above 60–65 mm. Hg. dyspnea becomes severe. In the range of 70–80 mm. Hg. the person becomes lethargic. Total anesthesia and death result with a level of 100-150 mm. Hg.

Continuing with the physiological classification on anoxia, Guyton has stated:

Hypohemoglobinemic anoxia, which results from anemia, carbon monoxide poisoning, or any other abnormality of hemoglobin transport, can receive a moderate benefit from oxygen therapy because the amount of oxygen transported in the dissolved form in the fluids of the blood can be greatly increased above normal even though that transported by the hemoglobin is hardly altered.
In ischemic anoxia, the value of oxygen therapy is still less because the problem here is sluggish flow of blood and not insufficient oxygen. However, even normal blood can carry about 10 per cent extra oxygen when the alveoli oxygen concentration is increased to 600 mm Hg. This 10 per cent difference may mean saving the life of a patient, as, for example, following an acute heart attack which causes the cardiac output to fall very low.14

Further comment can be made in regard to ischemic anoxia. Borden, Ebert, and Wilson reported that oxygen could prevent further necrosis to the ischemic area. They stated:

Thrombosis of a coronary artery leads to necrosis of the myocardium in the area supplied by this vessel. Adjacent to the necrotic muscle is viable but ischemic muscle, which gives rise to pain and represents a threat to the patient in that it, too, may undergo necrosis or give rise to ventricular fibrillation. One of the logical aims of therapy of myocardial infarction is to provide a more adequate supply of oxygen to the ischemic muscle until collateral circulation can develop. The inhalation of oxygen may accomplish this aim by increasing the oxygen content of the arterial blood so that each unit of blood carries more oxygen to the anoxic tissue. Oxygen therapy is also used to relieve dyspnea in myocardial infarction and to prevent the general deleterious effect of anoxia.15

Their study revealed some definite complications of myocardial infarction which were indicative of an oxygen need. Their conclusions were:

The purpose of this study was to define more accurately the indications for oxygen therapy in myocardial infarction. The results indicate that, in the patient without pulmonary edema or shock, the degree of anoxia is mild and of no importance to the body as a whole. The sole purpose of oxygen therapy in this group of patients would be the provision of greater


amounts of oxygen to ischemic areas in the myocardium. On the other hand, in those patients with evidence of pulmonary edema or shock, the anoxia may be severe. Oxygen therapy is mandatory to combat the general effects of anoxia on the body as well as to supply additional oxygen to the myocardium.\(^{15}\)

The heart itself may reveal characteristic manifestations of anoxia. Simonson and McGavack\(^{17}\) listed these as an apparent increase in excitability of myocardial tissue manifested in multifocal extrasystole, tachycardia, and, eventually, fibrillation.

The final classification of anoxia is listed as tissue utilization. Oxygen therapy is usually of very little benefit in this type of anoxia, since there is no abnormality of oxygen pickup by the lungs nor of transport to the tissues. Instead, the tissues need more oxygen than the enzymes can utilize.

From the review of literature, it was possible to classify the signs and symptoms of anoxia in the following manner:

1. Respiratory system
   a. Dyspnea
   b. Hyperpnea
   c. Hypoventilation
   d. Hyperventilation
   e. Stertorous breathing

2. Circulatory System
   a. Shock
   b. Tachycardia
   c. Arrhythmia
   d. Fibrillation
   e. Cyanosis
   f. Cardiac pain
   g. Pallor
   h. Changes in the acid-base balance of the blood

\(^{15}\) Borden, Ebert, and Wilson, op. cit.*, pp. 1370, 1371.

\(^{17}\) Simonson and McGavack (ed.), op. cit.*, p. 10.
3. Neurological System

a. Initial feeling of well-being
b. Restlessness
c. Mental depression
d. Hot flashes of the face
e. Headache
f. Frequent yawning or sighing
g. Roaring in the ears
h. Loss of judgment
i. Lack of fine muscle coordination
j. Vertigo
k. Fainting
l. Poor night vision
m. Muscle contractions and twitching
n. Convulsions
o. Loss of memory
p. Apprehension
q. Joint pain

4. Gastro-intestinal System*

a. Nausea
b. Vomiting

Oxygen Therapy

Following the discussion of the physiologic types of anoxia, the diseases or conditions which may produce anoxia, and the indications for oxygen therapy, it is appropriate to discuss oxygen therapy briefly. Oxygen therapy can be administered by several methods or procedures: by the use of a tent, a mask, a catheter, or cannula. The method chosen for administration depends upon the oxygen

*For purposes of this study, the signs and symptoms of nausea and vomiting were placed within the gastro-intestinal system, even though they were listed along with the neurological signs and symptoms in the medical literature. The reason for this classification was for clarification and to present these signs and symptoms in a system in which nurses usually associate them.
concentration desired. If possible, the wishes of the patient are also considered. Whatever method is used, the nurse has the responsibility of explaining the procedure and its purposes to the patient. Further, unless the hospital has an Inhalation Therapy Department, she is almost solely responsible to see that the equipment is working satisfactorily and that the desired concentration is being achieved. By use of a nasal catheter, concentrations of 30-50 per cent oxygen can be reached. Concentrations of up to 35 per cent can be achieved by nasal catheter and 55-60 per cent by tent. A concentration of 80-100 per cent can be obtained by use of the mask.

Excessive use of 100 per cent oxygen can cause fibrinolysis which may initiate excessive bleeding during surgery. Other toxic effects of the inhalation of pure oxygen are convulsions, edema of the lungs, and even death. Thus, it is evident that while oxygen therapy is very beneficial in certain conditions, it must be used judiciously.

Responsibility of Nurses in the Administration of Oxygen Therapy

Because the responsibility for the administration of oxygen therapy rests largely upon the nurse, and because many times the

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19Eugene E. Cliffton and Robert L. Clark, "Induction of Fibrinolysis by Hyperventilation with Oxygen and Carbon Dioxide," Angiology, XIV (June, 1963), p. 287.
necessity for its use is often left to her discretion, a survey was made of nursing textbooks to ascertain what preparational background is available to the professional nurse. The survey included textbooks in medical, surgical, and pediatric nursing, as well as in fundamentals of nursing. The selection was based on those areas of nursing in which the majority of p.r.n. orders for oxygen are carried out.

In reviewing the textbooks, all references referring to anoxia, hypoxia, and oxygen therapy were included. The texts in fundamentals of nursing were quite comparable in that generally only two or three signs and symptoms of anoxia were given. Some of the diseases and conditions in which anoxia could be manifested were mentioned. Several pages were then devoted to the methods of oxygen administration and the necessary safety precautions. For example, Montag and Swenson stated that "anoxia is characterized by cyanosis, dyspnea and rapid pulse." No other signs and symptoms were given. They listed the following conditions as those in which anoxia may occur: pneumonia, asthma, carbon monoxide poisoning, atelectasis, pulmonary edema, cardiac decompensation and coronary thrombosis. Fuerst and Wolff gave only one sign of anoxia — cyanosis. Harmer and


Henderson listed the conditions where anoxia may occur and also described some of the physiology of anoxia. Only one sentence was devoted to the signs and symptoms, but even this was somewhat more inclusive. They stated these as: increased pulse rate, rapid and shallow breathing, cyanosis, restlessness, headache, cardiac pain, and muscle twitching. Thirty-six pages were used in the discussion of the various methods and the procedure of oxygen administration.

Only a few signs and symptoms of anoxia were given in pediatric textbooks. Here, too, there was similarity among those which were listed. Benz gave quite a representative list of what was usually included as the signs and symptoms of anoxia. There were: dyspnea, flaring nostrils, cyanosis, and air hunger.

A survey of medical-surgical nursing texts did not reveal as much similarity in the listing of signs and symptoms of anoxia. For instance, although anoxia and hypoxia were referred to several times throughout the text by Smith and Gips, the only signs and symptoms given were cyanosis, dyspnea, and mental impairment. A few brief statements were made regarding the physiology of


respiration. Shafer, et al.\textsuperscript{25} included a section which described difficulties in breathing and on ventilation and stated that oxygen might be necessary. However, throughout the text, the only anoxic signs and symptoms given were dyspnea, cyanosis, deep and rapid breathing, and increased pulse rate. The most inclusive discussion of anoxic signs and symptoms was found in a text by Barbata, Jenson, and Patterson. They stated:

> Early signs of oxygen want (hypoxia) include an accelerated heart beat, rapid and shallow respirations, headache, retching or vomiting, restlessness, and muscle contractions or twitching. Change in the blood pressure at this time is not a good indication of the state or degree of oxygen want. As hypoxia increases, the patient may yawn or sigh repeatedly, and respirations may be noisy. The pulse rate gradually increases with the continued deprivation of oxygen. With severe oxygen want, the blood pressure may be elevated and the pulse rate extremely slow yet bounding in character. Progressive central nervous respiratory system depression occurs because of impaired oxygenation of the vital brain centers, and asphyxia results if immediate pulmonary resuscitation is not started.\textsuperscript{26}

Their discussion of oxygen administration stated that it was given for shock, cyanosis, and dyspnea. Though more inclusive, their discussion was still not complete. It was of interest to note that they included rapid and shallow respirations as a sign of anoxia, while Shafer, et al. included deep and rapid breathing. Harmer and Henderson also listed rapid and shallow breathing. Hypernea or

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increased depth of breathing was cited as a sign of anoxia in the scientific literature. Thus it appeared that there was some discrepancy in this particular area.

Overall, it was apparent that, in the nursing textbooks reviewed, the primary emphasis relative to oxygen therapy was on the procedures and not on the physiology involved nor on the signs and symptoms manifested by anoxia.

Summary

A review of scientific and nursing literature was made in order to provide information about anoxia. Normal respiratory physiology was reviewed as a basis for understanding the processes in respiration. This was followed by a discussion of how the normal processes of respiration can be altered with a resulting anoxic condition. The discussion included diseases and conditions in which anoxia may occur along with the manifestation of characteristic signs and symptoms. These overt and covert signs and symptoms can be classified according to their manifestations in certain body systems.

Those related to the circulatory system were:


The signs and symptoms identified as related to the respiratory system were:


Those identified along with neurological signs and symptoms but related to the gastro-intestinal system were:


Neurological signs and symptoms comprised the largest list and were the first detectable indications of anoxia. The signs and symptoms identified within this system were:

1. Initial feeling of well-being
2. Restlessness
3. Mental depression
4. Hot flashes of the face
5. Headache
6. Frequent yawning or sighing
7. Roaring in the ears
8. Loss of judgment
9. Lack of fine muscle coordination
10. Vertigo
11. Fainting
12. Poor night vision
13. Muscle contractions and twitching
14. Convulsions
15. Loss of memory
16. Apprehension
17. Joint pain

The types of anoxia in which oxygen therapy can be beneficial and a brief discussion of oxygen therapy were included.

Frequently the physician writes an order for oxygen to be given on a p.r.n. basis. From this order, the nurse must make the judgment of when to administer oxygen therapy. Since the administration of oxygen therapy is frequently the responsibility of
nurses, and the indications of a patient's oxygen need are left to their discretion, a survey was made of nursing textbooks to ascertain the available preparational background in this area. It was found that there was a very limited inclusion of the physiology of anoxia and that the list of anoxic signs and symptoms was far from inclusive. The emphasis was on methods and procedures for administration—the same as many technicians are being taught.

The problem of this study was to ascertain whether or not a selected group of professional nurses used scientific discrimination in the process of identifying overt signs and symptoms of anoxia upon which they then made the judgment to administer oxygen from a paramedical person's order.

The Method

The method chosen for the study was the descriptive survey because it is a way of obtaining facts about a current situation. According to Hillway, this method, also known as the narrative-survey and the status study, "attempts to describe a condition or to learn the status of something and, whenever possible, to draw valid general conclusions from the facts discovered."23 Good used the terminology—descriptive, narrative, status, and trend—as expressions of descriptive-survey investigation. So further stated:

CHAPTER III

METHODOLOGY

The problem of this study was to ascertain whether or not a selected group of professional nurses used scientific discrimination in the process of identifying overt signs and symptoms of anoxia upon which they then made the judgment to administer oxygen from a p.r.n. order.

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The terms survey and status suggest the gathering of evidence relating to current conditions. The expression 'normative' sometimes is applied to descriptive investigations, because the purpose is to determine the normal or typical condition or practice.29 However, he believed that the term "descriptive survey study" was more inclusive and appropriate.

Descriptive studies lend themselves to a wide range of techniques for the gathering of data. However, the procedures must be carefully planned because the aim is to obtain complete and accurate information.30

**Technique for Collection of Data**

Various techniques may be used to collect data for the descriptive survey: interview, questionnaire, observation, and survey-appraisal techniques. Some of these require direct judgment rather than some more objective form of evaluation. Good stated that those which require more judgment were the rating of specimens or items, the ranking of human beings, the comparison with scaled specimens, check lists with items which can be marked "yes" or "no," "present" or "absent," rating scales, and score cards.31

For purposes of this study, the check list seemed the most appropriate technique to ascertain the scientific discrimination used by nurses in determining anoxic conditions which warrant the

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31 Good, op. cit., pp. 336, 337.
use of oxygen therapy. The check list is a closed form type of questionnaire which provides "categorized data that greatly facilitate tabulating and summarizing processes."32 Also, the closed or fixed alternative questions "have the advantages of being 'standardizable,' simple to administer, quick and relatively inexpensive to analyze!"33

Development of the Tools for the Collection of Data

In order to collect data for the study it was necessary to develop several tools: a work sheet to categorize the signs and symptoms of anoxia; a cover letter to explain the study and elicit the cooperation of the respondents; and an instrument on which the participants could record their responses. Following a pretest to validate the check list and cover letter, a more extensive work sheet was needed on which to summarize the responses.

Work sheet.—After an extensive study of the scientific literature to determine the signs and symptoms presented by patients with various types of anoxia, it was apparent that a work sheet would be needed in order to organize the data. The literature indicated that these signs and symptoms were related to and could be categorized into the neurological, respiratory, circulatory and gastro-intestinal systems of the body. Therefore, in developing the work sheet, the

32 Good, op. cit., p. 277; Selltiz, loc. cit., p. 567.
33 Selltiz, loc. cit., p. 257.
signs and symptoms were divided into the above four systems or categories. (See Appendix A.) The signs and symptoms of anoxia and the distractor signs and symptoms were placed on the vertical left hand side of the work sheet. The top of the work sheet was numbered horizontally from one to one hundred, so that each respondent could be assigned a corresponding number. The body of the work sheet was divided so that check marks could be placed under each respondent's number according to the signs and symptoms which she had indicated. Provision was made for tallying the responses in the right hand vertical column of the work sheet.

Check list.—From the work sheet, a check list including the signs and symptoms of anoxia was developed. Distractor signs and symptoms were added so that scientific discrimination would be necessary in determining which were the actual signs and symptoms of anoxia. The distractor signs and symptoms were selected to fit into the same four categories. However, there was no categorization of any of the signs and symptoms in the check list itself. (See Appendix B.) The check list was arranged in two vertical columns on one sheet of paper. Preceding the check list was a brief statement of how anoxia was defined for this study, and the directions for the completion of the check list.

Cover letter.—A cover letter was developed in order to explain the study and elicit the cooperation of the respondents. It identified the investigator and stated that she had obtained permission to conduct the study. After use in the pretest, the necessary
minor changes were made. A revised copy of the cover letter as used for the study is found in Appendix B.

Pretest of the data collecting tool

Selltiz, et al., described the pretest and its purposes as follows: "The pretest is a try-out of the questionnaire to see how it works and whether changes are necessary before the start of a full-scale study."\(^{34}\) Another purpose or advantage of the pretest was given by Good. He stated: "Tabulation of the try-out responses in rough tables will indicate whether the answers can be tabulated satisfactorily and whether answers to the major questions are forthcoming."\(^{35}\) In this study, a pretest of the checklist was done in order to ascertain its practical use and to clarify any points of misunderstanding in the administration. In addition, the investigator wanted to determine the time, ease, and the emotional attitude elicited by taking the pretest.

Population of pretest.—The population of the pretest group consisted of eighteen students in a graduate program of nursing and included those in the medical-surgical specialty area. It was believed that this provided a somewhat comparable group with those nurses who would be used in the actual study. Permission was given by the Coordinator of the Medical-Surgical area to ask these students

\(^{34}\)Selltiz, et al., op. cit., p. 550.

\(^{35}\)Good, op. cit., p. 281.
if they were willing to participate in the pretesting of the check list. Twenty minutes was allowed during the last part of one of their regular class periods for the investigator to describe the study and elicit their cooperation. After eliciting their cooperation to participate and to offer constructive criticism about the check list and cover letter, the check list was administered to the group as a whole.

Findings of pretest.—Findings of the pretest revealed that it took the respondents from four to fifteen minutes to complete the check list. All of the eighteen respondents indicated that their cooperation was elicited by the cover letter and that they had felt assured of their anonymity. Sixteen indicated that the directions were clear and sufficient enough to enable them to proceed. Two of the respondents felt that the word "overt" might cause some problem since the signs and symptoms on the check list were not all overt. Four of the eighteen respondents felt that it was hard to make a decision when the signs and symptoms were listed without categorization on the check list. They would have preferred the signs and symptoms to have been grouped or listed in combinations.

Following the pretest, a more extensive work sheet was developed for compilation of the data. The work sheet was divided into four categories according to the circulatory, respiratory, gastrointestinal and neurological systems. Next, the signs and symptoms were grouped and placed in the appropriate category. It was then possible to tabulate the data.
A tabulation of data from the pretest indicated that the data lent itself to analysis. However, several of the distractor signs and symptoms were not checked, thus indicating that they were not eliciting real discrimination and were quite obviously not a sign or symptom of anoxia. Also, not all of the signs and symptoms were perceived as overt. Since respondents were asked to base their decisions to administer oxygen from a p.r.n. order upon the overt signs and symptoms of anoxia, all covert signs and symptoms were deleted from the check list. Further, the distractor signs and symptoms which were not checked at least once were eliminated. Since one of the purposes of the study was to determine whether professional nurses could recognize the signs and symptoms of anoxia relative to any disease or condition where oxygen therapy could be of benefit, it was decided not to group or classify the signs and symptoms, as suggested by some of the respondents on the pretest. After changes were made as indicated above, and minor changes were made in the wording of the directions, the check list was accepted for use in the study. After the check list had been accepted, approval to conduct the study was given by the chairman of the thesis committee.

The Study

Permission to conduct the study.—Initially, verbal permission to conduct the study was obtained from the Director of Nursing Service in a University Medical Center Hospital. The hospital had a bed capacity of 507 and was located in a large city. A formal letter of confirmation was written to the Director of Nursing Service. At a
second interview, the procedure for collection of the data was dis-
cussed. A copy of the letter which requested permission to conduct
the study is found in Appendix C.

Selection of the population.—The population of this study
consisted of professional nurses who were currently licensed by
the state to practice nursing. Since oxygen is used more frequently
in certain areas, it was decided to include nurses who were holding
staff positions on all three shifts of duty in the medical, surgical,
and pediatric units and in the intensive care units. A list con-
taining the names of all the professional nurses in these areas was
obtained from the nursing service office. An attempt was made to
contact individually the 145 nurses who composed the population of
those employed in these specified areas. Due to illness and vaca-
tion schedules, it was possible to contact only 126 members of this
population. As each nurse was contacted, a brief explanation of the
study was given and her willingness to participate was elicited. It
was emphasized that this would take approximately ten minutes of her
time. Nearly all expressed a willingness to complete the check
list; however, there were a few refusals, and some had to be approached
several times with a reminder to complete the questionnaire.

Setting of the study.—The study was conducted in a University
Medical Center Hospital. The hospital had a bed capacity of 437 and
was located in a large city in the Rocky Mountain area. In addition
to providing care for patients, the clinical facilities of the center
were used by several schools, namely, the schools of medicine, nursing, medical technology, x-ray technology, physical therapy, social work, and occupational therapy.

Collection of the data.—The data collection extended over a period of two and one-half weeks. For the majority of the respondents, the check list and directions were left with the nurse and picked up at the end of a two-hour period. At the completion of the study, 100 respondents, or 68 per cent of the total potential population of 145, had completed the check list.

The data were then transferred to a work sheet and prepared for analysis.

Approach to analysis.—Coding is the process by which data are categorized in order to facilitate analysis. A category set must meet certain basic rules. Selltiz, et al. listed these as:

1. The set of categories should be derived from a single classificatory principle.
2. The set of categories should be exhaustive; that is, it should be possible to place every response in one of the categories of the set.
3. The categories within the set should be mutually exclusive, it should not be possible to place a given response in more than one category with the set.

The categories in the work sheet were set up with these principles in mind. The data collected were transposed to categories corresponding to four of the body systems: circulatory, respiratory, gastro-intestinal, and neurological.

36 Selltiz, et al., op. cit., p. 392.
With the signs and symptoms classified into categories, it was possible to discuss the data in terms of percentage of the whole with relation to each category, and also with relation to each sign and symptom within the category. Tables were developed which listed the signs and symptoms included in the various categories of nurses who checked each item. This allowed for comparison of their judgments and the ascertaining of whether or not these judgments were based upon physiological and scientific principles. Since there were 100 respondents, the number and per cent are the same; therefore, only numbers will be used in the study to designate the responses.

Summary

In this chapter the method and techniques chosen for obtaining the data have been described. The descriptive-survey method with the check list as the data gathering technique was used for the study. The check list was used in order to require scientific discrimination by the respondent.

A pretest was done in order to clarify any points of misunderstanding in the administration or in the content. It also helped to determine whether or not the desired data was forthcoming. The results of the pretest indicated that covert signs and symptoms along with certain distractors should be eliminated. A few other minor changes were made in the wording of the directions and in the cover letter.
Permission to conduct the study was obtained from the Director of Nursing Service of a University Medical Center Hospital in the Rocky Mountain area. The population of the study consisted of professional nurses who were currently employed on all three shifts in the medical, surgical, pediatric, and the intensive care units of this hospital, which had a total bed capacity of 437.

The check list was given to the nurses on an individual basis and ample time was allowed for its completion. The data obtained from the study were classified, categorized, and compiled on a work sheet so that tabulation could be done.

An interpretation followed the presentation of each category analysis.

The problem was to ascertain whether or not a selected group of professional nurses used scientific discrimination in the process of identifying overt signs and symptoms of asxia upon which they then made the judgment to administer oxygen from a p.r.n. order.

The purpose of the analysis was to order the data in a manner that could be interpreted in a meaningful way. The explicit purposes of the analysis of the data for this study were: (1) to ascertain what signs and symptoms nurses used in making a decision to administer oxygen from a p.r.n. order; and (2) to ascertain whether or not nurses used scientific discrimination in making a decision to give oxygen therapy.

For purposes of clarity, the remainder of the chapter is presented in two parts: (1) presentation, analysis, and interpretation
CHAPTER IV

PRESENTATION, ANALYSIS, AND INTERPRETATION OF THE DATA

The data for this study were obtained through utilization of the descriptive survey, the check list having been used as the data collecting technique. The information was transposed into four categories which have been presented in tables as well as in a written analysis. An interpretation followed the presentation of each category analysis.

The problem was to ascertain whether or not a selected group of professional nurses used scientific discrimination in the process of identifying overt signs and symptoms of anoxia upon which they then made the judgment to administer oxygen from a p.r.n. order.

The purpose of the analysis was to order the data in a manner that could be interpreted in a meaningful way. The explicit purposes of the analysis of the data for this study were: (1) to ascertain what signs and symptoms nurses used in making a decision to administer oxygen from a p.r.n. order; and (2) to ascertain whether or not nurses used scientific discrimination in making a decision to give oxygen therapy.

For purposes of clarity, the remainder of the chapter is presented in two parts: (1) presentation, analysis, and interpretation.
of the signs and symptoms within each category; and (2) a comparison of the data related to the four categories.

Presentation, Analysis, and Interpretation of the Signs and Symptoms Within each Category

Data, as transposed from the check list, are presented for analysis and interpretation. The presentation includes the overt signs and symptoms of anoxia as well as the distractor signs and symptoms that were utilized for the study. A list of the overt signs and symptoms of anoxia which were included in the check list are given along with the bibliographical references in Appendix D.

The Respiratory System

Table I presents the responses of 100 professional nurses related to their judgment in giving oxygen from the overt signs and symptoms which they have indicated within the respiratory system.

Signs and symptoms of anoxia - respiratory system

Within the respiratory system four overt signs and symptoms of anoxia were identified for use in the study. Each of these is presented separately.

**Dyspnea.** Nearly all (ninety-six) of the respondents indicated that dyspnea was a sign of anoxia which would influence their decision to give oxygen therapy.

**Sterterous breathing.** Less than one-half (forty-three) of the respondents indicated that sterterous breathing could be a sign of anoxia.
TABLE 1

NUMBER OF RESPONSES BY ONE HUNDRED STAFF NURSES RELATIVE TO THE OVERT SIGNS AND SYMPTOMS OF ANOXIA AND THE DISTRACTORS SIGNS AND SYMPTOMS WITHIN THE RESPIRATORY SYSTEM USED AS A BASIS FOR THEIR JUDGMENT IN ADMINISTERING OXYGEN FROM A P.R.N. ORDER

<table>
<thead>
<tr>
<th>Respiratory System</th>
<th>Number of Respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signs and symptoms of anoxia</td>
<td></td>
</tr>
<tr>
<td>Dyspnea</td>
<td>96</td>
</tr>
<tr>
<td>Stertorous breathing</td>
<td>43</td>
</tr>
<tr>
<td>Hyperventilation</td>
<td>24</td>
</tr>
<tr>
<td>Increased depth of breathing</td>
<td>24</td>
</tr>
<tr>
<td>Distractor signs and symptoms</td>
<td></td>
</tr>
<tr>
<td>Retraction of the intercostal spaces</td>
<td>64</td>
</tr>
<tr>
<td>Shallow breathing</td>
<td>64</td>
</tr>
<tr>
<td>Perspiration</td>
<td>14</td>
</tr>
<tr>
<td>Bulging of the intercostal spaces</td>
<td>0</td>
</tr>
</tbody>
</table>

Interpretation of the data pertaining to the respiratory system

Dyspnea was the only sign within the respiratory system that was indicated with consistent frequency (ninety-six of the respondents). This is not surprising, since nearly every text included...
Hyperventilation. Less than one-fourth (twenty-four) of the respondents recognized hyperventilation as a sign of anoxia.

Increased depth of breathing. Twenty-four, or less than one-fourth, of the respondents indicated that increased depth of breathing was a sign of anoxia.

Distractor signs and symptoms

There were four respiratory distractor overt signs and symptoms intermingled with the overt signs and symptoms of anoxia. They are presented in the order of the number of responses given to each distractor sign and symptom.

Retraction of the intercostal spaces. Nearly two-thirds (sixty-four) of the respondents indicated that retraction of the intercostal spaces was a sign of anoxia.

Shallow breathing. Nearly two-thirds (sixty-four) of the respondents indicated that shallow breathing could be a sign of anoxia which would influence their decision in giving oxygen therapy.

Perspiration. Less than one-eighth (fourteen) of the respondents checked perspiration as a sign of anoxia.

Bulging of the intercostal spaces. None of the respondents checked this distractor as being a sign of anoxia.

Interpretation of the data pertaining to the respiratory system

Dyspnea was the only sign within the respiratory system that was indicated with consistent frequency (ninety-six of the respondents). This is not surprising, since nearly every text included
this sign in their discussion of anoxia. However, one would pose the question of whether or not this was memorization of knowledge rather than real scientific discrimination. Further, in view of the fact that many of the lay public equate shortness of breath with a need for more oxygen, it was not surprising that so many professional nurses would recognize this as a sign of anoxia. One might wonder why 100 per cent of the respondents did not indicate this as a sign of anoxia.

Stertorous breathing was the second most frequently indicated sign of anoxia in the respiratory system. However, one might say that this number of responses (less than 50 per cent) could have been the result of chance alone. Noisy respirations are frequently associated with the moribund, and this may have influenced some of the respondents to associate this sign with an oxygen need.

The signs of hyperventilation and increased depth of breathing were each recognized by less than one-fourth of the respondents. One could assume that neither of them is frequently observed with reference to their potential indication of an oxygen need. It was evident from the review of literature that there was a discrepancy regarding this latter sign as being a manifestation of anoxia. Two of the nursing textbooks reviewed indicated that shallow breathing was a sign of anoxia, while another nursing text maintained that deep breathing was a sign of anoxia. Deep breathing, and not shallow breathing, was found as a manifestation of anoxia in the scientific literature. Thus it can be assumed that this group of nurses was not
familiar with the scientific literature nor with some of the nursing literature. It was apparent that, except for dyspnea, the signs and symptoms of anoxia within the respiratory system were not frequently utilized by these respondents in making a decision to give oxygen therapy from a p.r.n. order.

Two of the distractor signs and symptoms elicited a response from nearly two-thirds of the respondents. The first of these was retraction of the intercostal spaces. Perhaps one explanation for so many responses to this sign is that on the pediatric units, nurses sometimes refer to a child as "retracting." However, in the literature reviewed, there was no physiological basis which indicated that retraction of the intercostal spaces was a sign of anoxia. The second sign which elicited this many responses was shallow breathing. It was not surprising to find that this many of the respondents did check shallow breathing since there was a discrepancy in the nursing literature. In view of the fact that anoxia stimulates the respiratory center in the medulla to accelerate the depth and rate of breathing, one would have to conclude that there was probably no scientific rationale used in this decision.

While perspiration frequently accompanies shock or a heart attack, it is not a physiological manifestation of anoxia. Since only fourteen of the respondents checked perspiration as a sign of anoxia, one could conclude that the other eighty-six did have the knowledge or were able to make the discrimination that this was not a sign of anoxia.
Because there were no responses to the distractor sign (bulging of the intercostal spaces) one could possibly conclude that discrimination had been used. On the other hand, the fact that retraction of the intercostal spaces was listed in consecutive order with bulging of the intercostal spaces on the questionnaire may have provided a basis for their decision, since it was rather obvious that a choice could be made between the two. (Sixty-four per cent chose the distractor sign, retraction of the intercostal spaces.)

Since the only source of oxygen intake is via the respiratory system, and since many of the overt signs and symptoms are of an observable nature, it is within this system that one would expect nurses to be most cognizant of the overt signs and symptoms or alterations resulting from an oxygen deficit. Yet, with the selected group of 100 professional nurses currently practicing nursing, there was no clear indication that a scientific rationale had been used as a basis for their judgment as to when oxygen therapy was indicated.

The Circulatory System

Table II presents the number of responses by 100 staff nurses relative to the overt signs and symptoms within the circulatory system which they indicated as a basis in making their decision to administer oxygen therapy when there is a p.r.n. order.

Signs and symptoms of anoxia - circulatory system

There were six overt signs and symptoms of anoxia included within the circulatory system. These are discussed as follows:
TABLE 2

NUMBER OF RESPONSES BY ONE HUNDRED STAFF NURSES RELATIVE TO THE OVERT SIGNS AND SYMPTOMS OF ANOXIA AND THE DISTRACTOR SIGNS AND SYMPTOMS WITHIN THE CIRCULATORY SYSTEM USED AS A BASIS FOR THEIR JUDGMENT IN ADMINISTERING OXYGEN FROM A P.R.N. ORDER

<table>
<thead>
<tr>
<th>Circulatory System</th>
<th>Number of Respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signs and symptoms of anoxia</td>
<td></td>
</tr>
<tr>
<td>Cyanosis</td>
<td>99</td>
</tr>
<tr>
<td>Shock</td>
<td>63</td>
</tr>
<tr>
<td>Increased pulse rate</td>
<td>56</td>
</tr>
<tr>
<td>Pallor</td>
<td>54</td>
</tr>
<tr>
<td>Pain in the cardiac area</td>
<td>51</td>
</tr>
<tr>
<td>Irregular pulse rate</td>
<td>24</td>
</tr>
<tr>
<td>Distractor signs and symptoms</td>
<td></td>
</tr>
<tr>
<td>Decreased blood pressure</td>
<td>46</td>
</tr>
<tr>
<td>Palpitation</td>
<td>13</td>
</tr>
<tr>
<td>Decreased pulse rate</td>
<td>13</td>
</tr>
<tr>
<td>Flushing of face</td>
<td>4</td>
</tr>
<tr>
<td>Increased blood pressure</td>
<td>4</td>
</tr>
</tbody>
</table>

A vast majority (ninety-nine) of the respondents indicated that cyanosis was a sign of anoxia. Almost two-thirds (ninety-three) of the respondents indicated that, in their judgment, shock was also a sign of anoxia. Nearly one-half (fifty-one) of the respondents indicated that an irregular pulse rate would influence their decision to administer oxygen from a P.R.N. order.

Cyanosis. A vast majority (ninety-nine) of the respondents indicated that cyanosis was a sign of anoxia.

Shock. Almost two-thirds (ninety-three) of the respondents indicated that, in their judgment, shock was also a sign of anoxia.

Pallor. Nearly one-half (fifty-one) of the respondents indicated that an irregular pulse rate would influence their decision to administer oxygen from a P.R.N. order.

Pain in the cardiac area. One-half (fifty-one) of the respondents indicated that a decreased pulse rate was a sign of anoxia.

Palpitation. Slightly over one-sixth (thirteen) of the respondents checked palpitation as a symptom of anoxia.

Decreased pulse rate. Approximately one-sixth (thirteen) of the respondents indicated that a decreased pulse rate was a sign of anoxia.
Cyanosis. A vast majority (ninety-nine) of the respondents indicated that cyanosis was a sign of anoxia.

Shock. Almost two-thirds (sixty-three) of the respondents indicated that, in their judgment, shock could be a sign of anoxia.

Increased pulse rate. Slightly over one-half (fifty-six) of the respondents indicated that an increased pulse rate would influence their decision to administer oxygen from a p.r.n. order.

Pallor. Slightly over one-half (fifty-four) of the respondents indicated that pallor was a sign of anoxia.

Pain in the cardiac area. One-half (fifty-one) of the respondents indicated that pain in the cardiac area was a symptom of anoxia.

Irregular pulse rate. Less than one-fourth (twenty-four) of the respondents indicated that an irregular pulse rate could be a manifestation of anoxia.

Distractor signs and symptoms

For the purposes of this study, five distractor signs and symptoms related to the circulatory system were included.

Decreased blood pressure. Nearly one-half (forty-six) of the respondents stated that a decreased blood pressure was a sign of anoxia.

Palpitation. Slightly over one-sixth (thirteen) of the respondents checked palpitation as a symptom of anoxia.

Decreased pulse rate. Approximately one-sixth (thirteen) of the respondents indicated that a decreased pulse rate was a sign of anoxia.
Flushing of the face. Only four of the 100 respondents indicated that flushing of the face could be a sign of anoxia.

Increased blood pressure. Of the 100 respondents, four indicated that an increased blood pressure could be a sign of anoxia.

**Interpretation of the data pertaining to the circulatory system**

Within the circulatory system, cyanosis was indicated as a sign of anoxia by ninety-nine of the respondents. This may be attributed to the fact that of the texts reviewed, every one stated that cyanosis was a sign of anoxia. This would be expected as the lay public are also familiar with cyanosis as being a sign of an oxygen need. Therefore it was felt that the response was due to a collection of facts by the respondents rather than to the use of scientific discrimination. This late sign of anoxia received more responses than any other sign or symptom used in the study; thus one might surmise that among these respondents the sign, cyanosis, was the most significant in affecting their decision to give oxygen therapy from a p.r.n. order. Should not professional nurses be able to identify earlier overt signs and symptoms so that the patient received oxygen therapy before he was in an advanced state of anoxia?

While in and of itself shock is a clinical entity, it was included as an overt sign of anoxia because it was so frequently included with the signs and symptoms of anoxia in both the scientific and the nursing literature. Because shock is associated with an inadequate or greatly impaired circulation of the blood and body fluids, and since it is the blood that carries oxygen, one could
assume that this would naturally arouse the thought of shock being associated with anoxia even with a minimal application of scientific principles. It was apparent that 63 per cent of the respondents did make this association.

Approximately two-thirds of the scientific and nursing literature indicated that an increased pulse rate was a sign of anoxia. It would seem that if the respondents utilized the available knowledge or used a scientific rationale, there should have been more than twenty-four responses to this sign of anoxia.

Pain in the cardiac area and pallor were each indicated by slightly over 50 per cent of the respondents. Pain in the cardiac area is caused by lack of oxygen to the cardiac muscle and very frequently occurs in conjunction with a myocardial infarction. If the respondents knew the physiology involved, one could expect to find that nearly 100 per cent would be able to recognize this symptom of anoxia. A characteristic pallor and cyanosis are frequently associated when there is an oxygen deficit. Yet a response by only one-half of the participants to pallor (and 99 per cent for cyanosis) does not disclose real scientific discrimination.

Among the distractor signs and symptoms included within the circulatory system, decreased blood pressure received the most responses (46 per cent). Blood pressure, per se, was not included in the discussion of the signs and symptoms of anoxia as reviewed in the scientific literature, and the nursing literature reviewed indicated that a change in blood pressure was not a good sign or
indicator of anoxia. It was, however, not surprising to find nearly one-half of the respondents indicating that it was a sign of anoxia, since low blood pressure is frequently associated with the clinical entity, shock. The remaining distractor signs and symptoms—palpitation, decreased pulse rate, flushing of the face, and increased blood pressure—each elicited only a few responses. This poses the possibility that more scientific discrimination was used in this area.

Of the six overt signs and symptoms of anoxia given within the circulatory system, five were indicated as such by over 50 per cent of the respondents. This indicates that the responses to these signs and symptoms of anoxia were slightly better than that to be expected by chance alone, and perhaps shows some knowledge in this area, but still very little scientific discrimination. The other sign of anoxia, an irregular pulse rate, elicited a response by less than one-fourth of the population of the study, thus indicating very little knowledge or scientific discrimination.

The Gastro-Intestinal System

Table III presents the number of responses by 100 staff nurses relative to the signs and symptoms of anoxia and the distractor signs and symptoms within the gastro-intestinal system used as a basis for their judgment in administering oxygen therapy from a p.r.n. order.

Signs and symptoms of anoxia - gastro-intestinal system

There were two overt signs and symptoms of anoxia within this system which were utilized for the study.
TABLE 3

NUMBER OF RESPONSES BY ONE HUNDRED STAFF NURSES RELATIVE TO THE OVERT SIGNS AND SYMPTOMS OF ANOXIA AND THE DISTRACTORS SIGNS AND SYMPTOMS WITHIN THE GASTRO-INTESTINAL SYSTEM USED AS A BASIS FOR THEIR JUDGMENT IN ADMINISTERING OXYGEN FROM A P.R.N. ORDER

<table>
<thead>
<tr>
<th>Gastro-Intestinal System</th>
<th>Number of Respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Signs and symptoms of anoxia</strong></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>6</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2</td>
</tr>
<tr>
<td><strong>Distractor signs and symptoms</strong></td>
<td></td>
</tr>
<tr>
<td>Abdominal cramping</td>
<td>5</td>
</tr>
<tr>
<td>Thirst</td>
<td>3</td>
</tr>
</tbody>
</table>
Nausea. Less than one-sixteenth (six) of the respondents indicated that nausea was a symptom of anoxia which could influence their decision in giving oxygen therapy.

Vomiting. Only two respondents indicated that vomiting could be a sign of anoxia.

Distractor signs and symptoms

Two distractor signs and symptoms were included that could be classified into the gastro-intestinal system.

Abdominal cramping. One-twentieth (five) of the respondents indicated that abdominal cramping was a symptom of anoxia.

Thirst. Three of the 100 respondents indicated that thirst was a symptom of anoxia.

Interpretation of the data pertaining to the gastro-intestinal system

It was quite apparent that the selected group of professional nurses generally do not use the signs and symptoms of nausea and vomiting as being those that could be indicative of anoxia.

There were practically as many responses from the distractor signs and symptoms as from the signs and symptoms of anoxia indicating that no scientific discrimination was made between the two. Since there were only a few responses to the signs and symptoms of anoxia as well as to the distractor signs and symptoms, it was concluded that this was probably due to a lack of both knowledge and scientific discrimination. It was evident that these respondents had very little knowledge regarding the manifestations of anoxia as related to the gastro-intestinal system.
The Neurological System

Table IV presents the number of responses by 100 staff nurses in regard to the overt signs and symptoms included in the neurological system upon which they would base their judgment in administering oxygen therapy from a p.r.n. order.

Signs and symptoms of anoxia - Neurological system

There were eleven overt signs and symptoms of anoxia within the neurological system which were utilized for the study.

**Apprehension.** Nearly two-thirds (sixty-two) of the respondents indicated that apprehension was a sign of anoxia.

**Restlessness.** Over one-half (sixty) of the respondents indicated that restlessness could be a manifestation of anoxia.

**Fainting.** Less than one-half (forty) of the respondents checked fainting as a sign of anoxia.

**Frequent yawning and sighing.** One-third (thirty-three) of the respondents recognized frequent yawning and sighing as a sign of anoxia.

**Convulsions.** One-third (thirty-two) of the respondents indicated that convulsions were a sign of anoxia.

**Dizziness.** One-fourth (twenty-five) of the respondents checked dizziness as a symptom of anoxia.

**Muscle contractions and twitching.** One-eighth (twelve) of the respondents indicated that muscle contractions and twitchings could be a manifestation of anoxia which would influence their decision in giving oxygen therapy.
<table>
<thead>
<tr>
<th>Neurological System</th>
<th>Number of Respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signs and symptoms of anoxia</td>
<td></td>
</tr>
<tr>
<td>Apprehension</td>
<td>62</td>
</tr>
<tr>
<td>Restlessness</td>
<td>60</td>
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<tr>
<td>Fainting</td>
<td>40</td>
</tr>
<tr>
<td>Frequent yawning and sighing</td>
<td>33</td>
</tr>
<tr>
<td>Convulsions</td>
<td>32</td>
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<tr>
<td>Dizziness</td>
<td>25</td>
</tr>
<tr>
<td>Muscle contractions and twitchings</td>
<td>12</td>
</tr>
<tr>
<td>Headache</td>
<td>10</td>
</tr>
<tr>
<td>Mental depression</td>
<td>9</td>
</tr>
<tr>
<td>Loss of memory</td>
<td>7</td>
</tr>
<tr>
<td>Roaring in the ears</td>
<td>2</td>
</tr>
<tr>
<td>Distractor signs and symptoms</td>
<td></td>
</tr>
<tr>
<td>Disoriented</td>
<td>26</td>
</tr>
<tr>
<td>Tingling -- extremities</td>
<td>17</td>
</tr>
<tr>
<td>Ringing of the ears</td>
<td>3</td>
</tr>
<tr>
<td>Frequent swallowing</td>
<td>2</td>
</tr>
</tbody>
</table>
Headache. One-tenth (ten) of the respondents indicated that a headache was one of the symptoms of anoxia.

Mental depression. Nine of the 100 respondents indicated that mental depression was a sign of anoxia that could influence their decision in administering oxygen therapy.

Loss of memory. Seven of the 100 respondents indicated that loss of memory was a manifestation of anoxia.

Roaring in the ears. Only two of the respondents indicated that roaring in the ears was a symptom of anoxia.

Distractor signs and symptoms

There were four distractor signs and symptoms included in the neurological system.

Disoriented. Approximately one-fourth (twenty-six) of the respondents indicated that the sign of being disoriented would influence their decision in giving oxygen therapy.

Tingling of the extremities. Approximately one-sixth (seventeen) of the respondents indicated that tingling of the extremities was a symptom of anoxia.

Ringing in the ears. Three of the 100 respondents indicated that ringing of the ears was a sign of anoxia.

Frequent swallowing. Of the 100 respondents, two indicated that the distractor sign, frequent swallowing, was a sign of anoxia.
Interpretation of the data pertaining to the neurological system

More signs and symptoms of anoxia may be found within this system than in any other system. Also, neurological signs and symptoms are the first apparent ones manifested in anoxia. Therefore, one might expect professional nurses to indicate considerable knowledge and scientific discrimination in this area.

Of the signs and symptoms of anoxia within this system, apprehension (62 per cent) and restlessness (60 per cent) were the two most frequently indicated by the respondents. These are signs and symptoms that could be elicited by several physiological disorders. One might expect, therefore, that this many responses were a result of a well calculated guess. Fainting, frequent yawning and sighing, convulsions, and dizziness were the next most frequently indicated signs of anoxia; and these were all indicated by considerably less than one-half of the respondents. Fainting and frequent yawning and sighing can occur in conjunction with cerebral anoxia. Both of these signs are often associated with a lack of oxygen by the lay public. Therefore, it could be expected that more than 40 per cent and 33 per cent respectively would be recognized by the respondents as a sign of anoxia. Convulsions, as manifested in Adams-Stokes syndrome, are caused by the lack of oxygen due to the apnea. This is only one of several conditions where convulsions are a sign of anoxia. Yet only one-third of the selected group of professional nurses indicated that convulsions were a manifestation of anoxia. Dizziness was checked as a sign of anoxia by one-fourth
of the respondents. One would expect that this symptom would have been associated with fainting and thus have elicited a similar number of responses.

The remaining signs and symptoms of anoxia within the neurological system elicited very few responses. For instance, headache was indicated by only 10 per cent of the respondents. According to the scientific literature, headache is quite frequently associated with anoxia. Thus it was apparent that the vast majority of this selected group of professional nurses did not utilize this early symptom and probably waited for a much more obvious manifestation of anoxia before administering oxygen therapy. It was obvious that muscle contractions and twitching, mental depression, loss of memory, and roaring in the ears were not utilized as signs of anoxia. Roaring in the ears is sometimes manifested in the anoxia associated with anemia. It was not surprising to find that only two would recognize it as a symptom of anoxia, since it was not frequently cited in the scientific literature.

Of the four distractor signs and symptoms, being disoriented, tingling of the extremities, ringing in the ears, and frequent swallowing, one-fourth or less of the respondents indicated each as a sign of anoxia. Of these, tingling of the extremities might be associated with tissue anoxia due to a tourniquet or some similar cause; however, the giving of oxygen therapy would be of no benefit in this type of anoxia. Decisions were to have been based on the overt signs and symptoms of anoxia which would respond physiologically to oxygen therapy. Since there was such an obvious lack in knowledge
of the characteristic manifestations of anoxia within the neurological system, one cannot conclude that only a few responses to the distractor signs and symptoms were really based on scientific discrimination.

It was of interest to note that, while the signs and symptoms of anoxia related to the neurological system comprised the largest list, there were eleven professional nurses who did not check any of the signs or symptoms within this category. (See work sheet, Appendix A.) Sixteen of the respondents checked only one sign or symptom, and of these sixteen, one had checked a distractor symptom. Another sixteen had checked only two signs and symptoms within this system, and one of these was also a distractor symptom.

Comparison of the Data as Related to the Four Categories

The review of literature for this study indicated that the signs and symptoms of anoxia could be categorized or classified according to four of the body systems: respiratory, circulatory, gastro-intestinal, and neurological.

Since it is via the respiratory system that the oxygen intake and carbon dioxide elimination take place, one would assume that nurses would especially associate the manifestations of anoxia with those overt signs and symptoms related to this system, and thereby would indicate more knowledge and scientific discrimination in this area. However, this assumption was not substantiated by the data. It was apparent that there was greater knowledge of the overt signs and symptoms of anoxia as related to the circulatory system, and
even here only slightly over 50 per cent indicated such knowledge. Even though the respondents were apparently more knowledgeable regarding the manifestation of anoxia as related to the circulatory system in comparison with the other systems, there was no indication that real scientific discrimination had been made in this system.

Overt signs and symptoms related to the gastro-intestinal system comprised the shortest list. Even so, by comparison, the overt signs and symptoms related to this system elicited fewer responses to either the signs and symptoms of anoxia or to the distractor signs and symptoms than was elicited by those in any of the other three categories.

Compared to the other three systems, the overt signs and symptoms of anoxia related to the neurological system comprised the longest list. Since nearly all of the scientific literature clearly stated that early signs and symptoms of anoxia were first apparent within the neurological system, one would have expected to find that professional nurses would be quite discriminatory in this area. In detecting the early overt signs and symptoms of anoxia the nurse should be in a better position to make an appropriate judgment as to when to give oxygen therapy from a p.r.n. order and thereby greatly contribute to the welfare of her patient. From the responses it was apparent that no more discrimination or knowledge was used regarding the signs and symptoms of anoxia as related to the neurological system than to those of any other system. In fact, there was an indication of less knowledge related to this system than that related to the circulatory system. Thus while one might expect the
respondents to reveal the most knowledge and use of a scientific rationale in regard to the overt signs and symptoms of anoxia as related to the respiratory and the neurological system, it was evident that this was not so. The data indicated that this group of respondents displayed more knowledge of the manifestations of anoxia as related to the circulatory system than to any other system.

Summary

This chapter has presented, analyzed, and interpreted the data as collected for the study. The first part of the chapter included a presentation, analysis, and interpretation of the overt signs and symptoms as related to each category or body system. It was found that dyspnea and cyanosis were the most frequently indicated signs of anoxia (96 and 99 per cent, respectively). This response was expected on the basis of knowledge alone because of the inclusion of these signs in all of the scientific and the nursing literature and because of the fact that the lay public equates these signs with a need for oxygen. With the exception of those responses related to the circulatory system, there was no consistency in the responses to the signs and symptoms of anoxia related to the other systems which would indicate that a thorough knowledge or scientific discrimination had been used.

The second portion of the chapter consisted of a comparison of the data of the four categories. From the review of literature it could be expected that the respondents would demonstrate the most
knowledge and scientific discrimination in regard to the overt signs and symptoms of anoxia as related to the respiratory and the neurological systems. However, the data revealed that there was more knowledge evidenced in regard to the manifestation of anoxia within the circulatory system. There was the smallest proportion of responses made in regard to the overt signs and symptoms within the gastro-intestinal system. None of the categories elicited responses that would depict real scientific discrimination.

This chapter presents a summary of the study and recommendations that were based on the data obtained for the study, and recommendations for further related investigations.

The problem was to ascertain whether or not a selected group of professional nurses used scientific discrimination in the process of identifying overt signs and symptoms of anoxia upon which they then made the judgment to administer oxygen from a p.r.n. order.

The purposes of the study were: (1) to identify the signs and symptoms of anoxia which are based on scientific physiological principles; (2) to ascertain what overt signs and symptoms professional nurses used in making a decision to administer oxygen from a p.r.n. order; (3) to determine if certain systemic categories of signs and symptoms were recognized more frequently than others; (4) to ascertain whether or not professional nurses used a scientific discrimination in making a decision to give oxygen therapy.

A review of scientific and nursing literature was made in order to ascertain the physiology of anoxia, its signs and symptoms, and the treatment. The review of nursing literature revealed that
CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This chapter presents a summary of the study, conclusions that were based on the data obtained for the study, and recommendations for further related investigations.

Summary

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A review of scientific and nursing literature was made in order to ascertain the physiology of anoxia, its signs and symptoms, and the treatment. The review of nursing literature revealed that
there was a very limited inclusion of the physiology of anoxia and that the list of anoxic signs and symptoms was far from inclusive.

The method chosen for the study was the descriptive-survey. The study was conducted in a University Medical Center Hospital in a large metropolitan area. The population of the study consisted of professional nurses who held staff positions on the general medical, surgical, and pediatric units and the intensive care units.

The data were collected over a period of two and one-half weeks during which time an attempt was made to contact individually each nurse that could comprise the population of the study. Of the total potential population (145), 100 completed the check list for use in the study. The data were transposed to a work sheet and were separated into categories according to the respiratory, circulatory, gastro-intestinal, and the neurological systems of the body. This made it possible to tabulate the data and facilitate the analysis. Tables were presented for each category. The data were analyzed, interpreted, and conclusions drawn.

Conclusions

Since the population of the study was limited to a selected group of professional nurses in only one hospital setting, broad generalizations cannot be made. However, on the basis of the data obtained in this study, the following conclusions were made:

1. Since only two well-known and late overt signs and symptoms (cyanosis and dyspnea) were identified with consistent frequency by the respondents, this group of professional nurses
was not aware of the many overt signs and symptoms of anoxia which warrant the use of oxygen therapy.

2. There was apparently a lack of knowledge and scientific discrimination regarding the overt signs and symptoms of anoxia as manifested in the body systems, with the exception of those related to the circulatory system. This knowledge of the manifestations of anoxia within the circulatory system (by slightly over 50 per cent of the respondents) still did not indicate that decisions were based on scientific discrimination, or there would have been a much higher percentage of responses to the overt signs and symptoms of anoxia as classified within this system.

3. Finally, that because of this lack of knowledge and scientific discrimination, many patients might be placed at a disadvantage or even in jeopardy by not receiving oxygen therapy when it is indicated.

Recommendations

As a result of this study, the following recommendations were made:

1. That this same study be repeated in another similar setting with a population of professional nurses as an attempt to verify the findings of this study.

2. That another type of data-gathering tool be developed for ascertaining the scientific discrimination used by professional nurses in determining an oxygen need. This would either substantiate
the conclusions of the study or perhaps point out certain weaknesses in the instrument as used for this study.

3. That this study be extended to include the covert as well as the overt signs and symptoms of anoxia used in the process of determining whether or not a patient needs oxygen therapy.

4. That the curricula of schools of professional nursing be studied to ascertain whether or not the physiological basis for and the signs and symptoms of anoxia are being taught.

Further recommendations were:

1. That the check list as utilized for the study be given to a similarly selected group of practical nurses in order to compare their responses with the data obtained from the selected group of professional nurses.

2. That an observational study of the signs and symptoms demonstrated by anoxic patients be done to verify if the stated decisions of the nurses are consistent with their nursing practice.
APPENDIX A

Work Sheet
Dear Participant:

I am undertaking a study to ascertain the signs and symptoms that professional nurses use to determine an anoxic condition in the patients for whom she is caring and upon which she then makes the decision of whether or not to give oxygen from a p.r.n. order.

This will take approximately ten minutes of your time. Directions and a check list for your convenience in supplying the information are found on the following pages.

The information which you supply will be completely confidential and your name will not be used in any way during my study.

If you wish to know the findings of my study, a summary will be given to Mrs. Brunckow upon the completion of my thesis.

Your assistance in helping me obtain the data I need will be greatly appreciated.

Sincerely yours,

(Mrs.) Hazel Rice

Mrs. Hazel Rice is a graduate student in the Department of Nursing, University of Colorado Graduate School and has our permission to do this study. Your cooperation in helping her obtain the data for the study will be appreciated.

Smith V. Olsen
Chairman, Thesis Committee
Dear Participant:

I am undertaking a study to ascertain the signs and symptoms that professional nurses use to determine an anoxic condition in the patients for whom she is caring and upon which she then makes the decision of whether or not to give oxygen from a p.r.n. order.

This will take approximately ten minutes of your time. Directions and a check list for your convenience in supplying the information are found on the following pages.

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__________________________
Edith V. Olson
Chairman, Thesis Committee
NURSE DECISIONS IN GIVING OXYGEN THERAPY

Frequently oxygen therapy is ordered on a p.r.n. basis. It then becomes the responsibility of the nurse to determine whether her patient presents an anoxic condition.

For purposes of this study, the term, anoxia, is broadly used to imply any oxygen deficiency in the body which is sufficient enough to give rise to overt signs and symptoms and thus warrant the use of oxygen therapy.

Directions: Please read the following list of signs and symptoms carefully and then go back and check those overt signs and symptoms which you believe are indicative of anoxia and would provide a basis for your decision to administer oxygen from a p.r.n. order.
<table>
<thead>
<tr>
<th>OVERT SIGNS AND SYMPTOMS</th>
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<tr>
<td>Hyperventilation</td>
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<tr>
<td>Cyanosis</td>
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<tr>
<td>Increased blood pressure</td>
</tr>
<tr>
<td>Decreased blood pressure</td>
</tr>
<tr>
<td>Flushing of face</td>
</tr>
<tr>
<td>Nausea</td>
</tr>
<tr>
<td>Vomiting</td>
</tr>
<tr>
<td>Mental depression</td>
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<tr>
<td>Apprehension</td>
</tr>
<tr>
<td>Irregular pulse</td>
</tr>
<tr>
<td>Roaring in ears</td>
</tr>
<tr>
<td>Ringing in ears</td>
</tr>
<tr>
<td>Dyspnea</td>
</tr>
<tr>
<td>Increased pulse rate</td>
</tr>
<tr>
<td>Decreased pulse rate</td>
</tr>
<tr>
<td>Pain - cardiac area</td>
</tr>
<tr>
<td>Headache</td>
</tr>
<tr>
<td>Shallow breathing</td>
</tr>
<tr>
<td>Increased depth of breathing</td>
</tr>
</tbody>
</table>
1301 E. Bates Avenue  
Englewood, Colorado 80110  
May 3, 1965

Mrs. ________________________
Director of Nursing Service

Dear Mrs. ______________________

As a graduate student in nursing at the University of Colorado, I wish to conduct a research study on nurses' judgments in administering oxygen from a p.r.n. order.

In general, my thesis problem concerns the signs and symptoms of anoxia, and whether or not nurses can recognize and use scientific discrimination in identifying these signs and symptoms. I feel a need is indicated for this study because of the lack in the nursing literature of complete lists of signs and symptoms of anoxia and the scientific principles involved.

In order to do the study, I need a questionnaire to be filled out by nurses practicing in the medical, surgical, pediatric and intensive care units. May I have your written permission to ask the nurses employed in these areas to participate in filling out a questionnaire?

Sincerely yours,

(Mrs.) Hazel Rice

Mrs. Hazel Rice has our approval to pursue this problem for her thesis.

Edith V. Olson
Chairman, Thesis Committee
May 3, 1965

Mrs. __________
Director of Nursing Service

Dear Mrs. __________:

As a graduate student in nursing at the University of Colorado, I wish to conduct a research study on nurses' judgments in administering oxygen from a p.r.n. order.

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In order to obtain data for my study, I need to administer a questionnaire to professional nurses who are currently practicing in the medical, surgical, pediatric and intensive care units. May I have your written permission to ask the nurses employed in these areas to participate in filling out a questionnaire?

Sincerely yours,

(Mrs.) Hazel Rice

Mrs. Hazel Rice has our approval to pursue this problem for her thesis.

Edith V. Olson
Chairman, Thesis Committee
Overt Signs and Symptoms of Anoxia Used for the Study

**Respiratory System**

**Dyspnea**


**Stereotomic Breathing**


**Hyperventilation**


**Increased depth of breathing (hypopnea)**


**Circulatory System**

**Gyanoceia**

RESPIRATORY SYSTEM

Dyspnea


Sterterous Breathing


Hyperventilation


Increased depth of breathing (hyperpnea)


CIRCULATORY SYSTEM

Cyanosis

Shock


Increased pulse rate


Pallor

Valdivia, loc. cit.

Pain — cardiac area


Irregular pulse rate


GASTRO-INTESTINAL SYSTEM

Nausea

Barbata, op. cit., p. 124; Lyght, op. cit.; p. 1417; and Valdivia, op. cit.

Vomiting

Barbata, op. cit., p. 124; Lyght, loc. cit.; and Sodeman, op. cit., p. 613.
NEUROLOGICAL SYSTEM

Apprehension


Restlessness

Barbata, op. cit., p. 124; Harmer, op. cit., p. 768; and Gilbert, loc. cit.

Fainting


Frequent yawning and sighing

Barbata, op. cit., p. 125; and Gilbert, op. cit., p. 12.

Convulsions


Dizziness

MacBryde, op. cit., p. 689; and Valdivia, op. cit., p. 77.

Muscle contractions and twitchings

Guyton, loc. cit.; Harmer, op. cit., p. 768; and Lyght, op. cit., p. 1417.

Headache

Guyton, loc. cit.; Lyght, loc. cit.; and Sodeman, op. cit., p. 881.

Mental depression

Guyton, loc. cit.; Sodeman, op. cit., p. 613; and Valdivia, op. cit., p. 77.

Loss of memory

Gilbert, op. cit., p. 12; and Guyton, loc. cit.

Roaring in the ears

Lyght, op. cit., p. 33.
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BIBLIOGRAPHY

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THE PROPERTIES OF AN ATTENUATED STRAIN OF
VACCINIA VIRUS

by

T. Jacob John

M.B., B.S., University of Kerala, 1959

A Thesis submitted to the Faculty of the Graduate
School of the University of Colorado in partial
fulfillment of the requirements for the Degree

Master of Science

Department of Microbiology

1966
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This Thesis for the M.S. degree by

T. Jacob John has been approved for the comparison with those of the standard Department of Microbiology and Lederle-Chinoin laboratories (116). In primary passage monkey kidney (PMK) and HEp 2 cells CV I consistently produced smaller plaques than strain 17, which in turn produced smaller plaques than CEF (CEF) however, the above ordering was reversed, and of multiplications of the three strains were assayed in HE cells, demonstrating their role in the determination of virulence. Laminarin membrane of chick embryos, CV I produced smaller pocks than the others. Histologically more dense cellular infiltration was seen in CV I pocks. Also this strain was found to be more virulent for the embryos. Greater virulence also was found for young mice, when injected intracranially. Again, compared with the other two strains, CV I multiplied faster in mouse-brain, thereby explaining the increased virulence. Larger amounts of interferon were induced by
The Properties of an Attenuated Strain of Vaccinia Virus

Certain biological characteristics of the CV I strain of vaccinia virus have been studied and compared with those of two standard strains, namely CL (117) and Lederle-Chorioallantoic (118). In secondary rhesus monkey kidney (MK) and HEp 2 cells CV I consistently produced smaller plaques than strain 117, which in turn produced smaller plaques than 118. In chick embryo fibroblasts (CEF) however, the above order was reversed. Rates of multiplication of the three strains were assayed in MK cells, demonstrating their role in the determination of plaque sizes. On the chorioallantoic membrane of chick embryos, CV I produced smaller pocks than the others. Histologically more dense cellular infiltration was seen in CV I pocks. Also this strain was found to be more virulent for the embryos. Greater virulence also was found for young mice, when injected intracranially. Again, compared with the other two strains, CV I multiplied faster in mouse-brain, thereby explaining the increased virulence. Larger amounts of interferon were induced by
strain 118 than by the other two, and all three strains were relatively insensitive to plaque reduction by interferon.

However, in MK cells, strain CV I showed a markedly increased sensitivity to an antiviral thiosemicarbazone, a property suitable for a marker. The difference in sensitivity was not great in CEF.

This abstract of 200 words is approved as to form and content.

Signed Vincent A. Fulginiti, M.D.
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The Properties of an Attenuated Strain of Vaccinia Virus

Foreword

Even though vaccination against smallpox is a relatively safe procedure, it is not altogether free from risk. (1). Complications of vaccination may range from the trivial and inconsequential accidental inoculation to the incapacitating and often fatal encephalitis or progressive vaccinia. (1). Since most of the complications follow the first exposure, the need exists for a safer vaccine, especially for primary vaccination. (2). Inactivated vaccinia virus has not, in general, been found to be sufficiently antigenic. (3). Therefore the search has been for attenuated strains of vaccinia. (2,4).

Rivers, in the 1930's, studied two strains of tissue culture adapted vaccinia virus, designated the First revived and the Second revived strains. (5). They were later called CV I and CV II respectively by Parker. (6). Upon inoculation in the rabbit's or human skin they produced milder lesions than those caused by their parent strain. (5). Also the immunity to re-vaccination was short-lived. (5,7). Therefore they were justifiably considered not to afford adequate protection against smallpox, and interest in them died down.

The recent renewal of interest in them stems from the need
for protection against the complications of vaccination, which, in the United States and the Netherlands at least, have taken a much higher toll than smallpox itself for the past several decades. (2,4).

The CV II strain has successfully been used in the Royal Netherlands Army, resulting in a marked reduction in their high incidence of encephalitis following primary vaccination. Certain properties of this strain have been studied by van der Noordaa. (4). In the following pages are recorded the results of investigations done primarily to determine certain properties of the CV I strain. They have been compared with those of two standard strains of vaccinia.

These investigations were done in the laboratory of C. Henry Kempe and Vincent A. Fulginiti, under the advisorship of Fulginiti. Simone Douard rendered her assistance in the preparation of tissue cultures and Iris Abraham helped in the measurement of thiosemicarbazone. Much interest in this work was shown by J. van der Noordaa. I am indebted to them all.
Chapter 1

Three Strains of Vaccinia

The primary purpose of these investigations was to study the properties of the CV I strain. However, since this strain seemed to behave differently on the human skin from the usual vaccine strain(s), some comparative studies were conducted. This chapter describes two such standard strains studied and summarises the history of CV I.

Strain CL. (ATCC. V.R. 117)

This strain of vaccinia virus was obtained from the American Type Culture Collection, who received it from Fenner. (8,9). It was probably derived from the New York City Board of Health strain which originally came from England. (6). It was propagated and maintained at the Connaught Laboratories, thus deriving its strain designation of CL. After several passages in the rabbit's skin and testes, it underwent 17 passages on the chorioallantoic membrane (CAM) of embryonated hen's eggs, including two single pock passages. (9). It is designated as an International type strain. (8). Obtained as lyophilized CAM-grown virus, it was passed once in chick embryo fibroblasts and a pool was made for all subsequent experiments. In the following pages, it is referred to as strain 117.
Strain Lederle - Chorioallantoic. (ATCC, V.R. 118)

This strain also was obtained from the American Type Culture Collection, who received it from the Lederle Laboratories, American Cyanamid Company. (8). It was derived from the New York City Board of Health strain, maintained at the Lederle Laboratories since 1909 by alternate passages in calves, rabbits and man. This strain is used for the production of calf-lymph vaccine in the United States. (8). In 1949 it underwent five serial passages on CAM. Obtained as lyophilised, CAM-grown virus, it was passed once in chick embryo fibroblasts and a pool was made for all further investigations. Hereafter it is referred to as strain 118.

River's First revived strain. (CV I)

With the purpose of producing bacteria free vaccine, Rivers began propagating vaccinia virus in chick embryo tissue culture, in 1930. (5). The original inoculum was the New York City Board of Health strain. After several passages in tissue culture, the titer, measured by intracutaneous inoculation in rabbits, was seen to drop. In order to enhance the titer, he passed the 34th tissue culture passage material in rabbit testes, six times. (5). He called this strain the First revived strain, which was again passed 31 times in tissue culture, before a second revival of virulence for rabbit's skin was performed. (5).
Kempe received the CV I strain from Parker, in 1948. In their hands, it underwent approximately 78 serial passages on chick embryo chorioallantoic membrane. (10). Afterwards it was supplied to the Wyeth Laboratories who are currently engaged in the preparation of lyophilised chorioallantoic membrane-grown vaccinia virus vaccine. This vaccine is now under experimental investigation by Kempe and associates who are vaccinating adults and children, especially those with eczema. Until recorded information is available, it may be said that this vaccine seems to produce smaller skin lesions and milder general reactions than the calf-lymph vaccine available commercially. An example of the vesicle, nine days after primary vaccination by multiple pressure, using CV I, is shown in Figure 1. The vesicle measured 5 mm. in diameter.

The lyophilised vaccine was reconstituted, grown once in chick embryo fibroblasts and a pool was made for all further experiments.
Figure 1. Primary vaccination by multiple pressure using CV I. The vesicle on the 9th day.
Chapter 2

Growth in Tissue Culture

The plaque morphology and size and the rates of multiplication of the strains CV I, 117 and 118 were compared in tissue culture, in an attempt to explain the low virulence of CV I for man. Similar investigations have been done with the CV II strain, but not with CV I.

Materials and Methods:

Suspensions of rhesus monkey kidney cells were obtained commercially. (Flow Laboratories.) They were grown at 37°C and maintained at 33°C. The primary cell monolayers were trypsinised and secondary cells were grown in 35 mm. x 10 mm. plastic petri dishes, (Falcon Plastics) at 37°C in a 5% CO₂ atmosphere. Each batch of primary cells was checked for contaminating, hemadsorbing viruses and none was found. (11).

A line of HEp 2 cells have been maintained in this laboratory for several years. It was grown and maintained in similar petri dishes at 37°C in the CO₂ incubator.

Chick embryo fibroblasts (CEF) were prepared from 10 or 11 day-old embryos, essentially according to previously described methods. (12).

The growth and maintenance media of these cells are described in the appendix. Since the non-fetal bovine serum...
was found to contain nonspecific inhibitors, the maintenance medium was modified by substituting fetal calf serum for bovine serum when it was used for diluting the virus or incorporating in the agar overlay. Also phenol red was omitted.

For the production of plaques, 0.2 mls. of serial ten-fold dilutions of the virus was inoculated in duplicate on to the cell monolayers in petri dishes. The maintenance medium in MK, CV I and CV II cells, and at 72 hours in the former two, was sucked off, or the growth medium was removed and the cells washed once with sterile saline, before inoculation. The dishes were returned to the CO₂ incubator, rocked every half hour, and agar medium added after four hours. This agar overlay consisted of maintenance medium containing 0.6% (W/V) agar (purified, Difco), and no phenol red, at pH 7 and temperature of 41° C. A similar agar overlay, containing 0.01% neutral red was added about 4 hours before examining and counting the plaques. With an adapter and a slide projector, the dishes were projected at 20x magnification, and the diameters of ten largest plaques were measured for each dish.

The rates of multiplication of the three strains were compared in secondary monkey kidney (MK) cells. Petri dishes were inoculated with 13 to 40 plaque forming units (PFU), incubated, and duplicate dishes withdrawn at 6, 12, 24, 33,
48, 55 and 72 hours. The cells were scraped with rubber policemen and the contents of duplicate dishes were pooled and stored frozen at -70°C. About three weeks later they were titrated, the first five harvests in one run and the last two in another.

Results:

The average plaque diameters were measured at 48 hours, in MK, HEp 2 and CEF cells, and at 72 hours in the former two. The results are shown in Table 1.

The plaque diameters of each strain varied between experiments in the same tissue. However, in MK and HEp 2 cells, CV I always produced the smallest, 118 the largest and 117 the intermediate plaques. In CEF the order was reversed, CV I producing the largest and 118 producing the smallest plaques. Experiments 1 and 5 were performed simultaneously using inocula from the same master dilutions and using identical agar overlay. The plaques in MK were much larger than those in CEF, and the ratios between plaque diameters in MK and CEF were 1.4 for CV I, 2.36 for 117 and 3.09 for 118.

Certain differences in plaque morphology were observed. These were most marked in MK and least in CEF. In MK and HEp 2 cells, the plaques of strains 117 and 118 were more uniformly circular than those of CV I. Also the margins of the former two were stained deeper making the plaque
boundaries better defined than those of CV I. These differences in plaque size and morphology are illustrated in Figure 2.

The rates of virus multiplication are shown in Figure 3. The multiplication has been expressed as a function of the inoculum. At every interval measured, 118 had the fastest and CV I the slowest rates. Strain 117 fell in between.

Experiments 1 and 5 in Table 1 were performed using identical inocula in MK and CEF cells. However, the resultant numbers of plaques were not similar for strains 117 and 118 in MK and CEF. These results are shown in Table 2.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Number of plaques*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV I</td>
</tr>
<tr>
<td>MK</td>
<td>106</td>
</tr>
<tr>
<td>CEF</td>
<td>111</td>
</tr>
</tbody>
</table>

*Average from duplicate dishes.

Table 2. The number of plaques produced by identical inocula in MK cells and CEF.

Compared with the number of plaques in MK, the efficiency of plaque formation in CEF was 100% for CV I, 38% for 117 and 36% for 118. Another experiment also produced similar results.
<table>
<thead>
<tr>
<th>Experiment No</th>
<th>Plaque diameters at 48 hrs. in MK</th>
<th>Plaque diameters at 72 hrs. in MK</th>
<th>Plaque diameters at 48 hrs. in HEP 2</th>
<th>Plaque diameters at 72 hrs. in HEP 2</th>
<th>Plaque diameters at 48 hrs. in CEF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV I 117 118</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>46.2 55.8 65.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>27.6 38.2 40.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>35.5 45.9 52.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6.4 22.7 25.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>14.4 28.2 33.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5**</td>
<td>33 23.6 21.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>26.6 21.2 19.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Petri dishes stained at 48 hours and incubated another day.
**Experiments 1 and 5 were done simultaneously.

Table 1. Average plaque diameters of CV I, 117 and 118 measured in mm. at 20x magnification.
Figure 2. Plaques by strains 118, 117 and CV I in MK cells. (Not magnified)
Figure 3. The rates of multiplication of strains 118, 117 and CV I in MK cells.
Discussion:

Compared with unattenuated (wild) measles virus, the attenuated Edmonston strain produces smaller plaques in BSC-1 cell line, and multiplies at slower rates in primary cynomolgus monkey kidney cells. (13, 14). Similarly, CV II, an attenuated vaccinia virus forms smaller plaques and multiplies slower than two European vaccine strains (Elstree and Copenhagen) in two human cell lines. (4). Therefore, it is not surprising that CV I produced smaller plaques than strains 117 and 118 in HEp 2 and MK cells. However, at this juncture, contamination by mycoplasma was discovered in HEp 2 cells and further studies were abandoned in them.

The multiplication rates of the three strains paralleled the plaque sizes, once again suggesting the role of growth rate in the production of plaque size. Thus strain 118 multiplied fastest and produced the largest plaques, CV I multiplied slowest and produced the smallest plaques, and strain 117 fell between in both respects.

Since CV I has been passed several times in embryonic chick tissues and adapted for growth in it, the three strains were compared in CEF also. All strains produced smaller plaques in CEF than in MK cells. However, CV I produced the largest, 117 the intermediate and 118 the smallest plaques.

The efficiency of plaque formation of strains 118 and 117 in
CEF were only 36 to 38% of that in MK cells; CV I produced equal numbers of plaques in both cells. The ratios of plaque diameters in MK to those in CEF cells were 1.4 for CV I, 2.36 for 117 and 3.09 for 118. For these reasons it may be concluded that all three strains grow better in MK cells than in CEF, but among the three, CV I is the most efficient for growth in CEF.

The intermediate position held by strain 117 in the above experiments may perhaps be due to the fact that it has undergone 18 passages as against 6 passages of 118, in embryonic chick tissues. This would suggest that attenuation varies according to the passage level in these tissues. This is indeed true with measles virus which was attenuated by adaptation in chick cells, and further attenuated by 77 additional passages. (15, 16). It may be expected therefore, that further attenuation of CV I may be achieved by additional passages in chick cells.

Apart from the comparative plaque sizes, the plaque morphology also offers itself as a marker for CV I. In MK and HEp 2 cells CV I plaques could easily be distinguished by their less distinct borders when stained with neutral red.

Summary:

In secondary monkey kidney (MK) and HEp 2 cells CV I
produced the smallest, 117 the intermediate and 118 the largest plaques. The order of plaque sizes was the same as the order of the rates of multiplication in MK cells. The plaque borders of 117 and 118 stained deeper with neutral red, but not those of CV I. In chick embryo fibroblasts (CEF) the order of plaque sizes were reversed. Compared with the plaquing efficiency in MK cells, that in CEF was 100% for CV I, and 36 to 38% for 118 and 117.
no pocks were seen. Table 3 illustrates this toxicity or increased virulence of CV I over the other strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Inoculum*</th>
<th>No of eggs Inoculated</th>
<th>No. alive at 48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>117</td>
<td>10</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>118</td>
<td>10</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>CV I</td>
<td>10</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

*Approximate number of PFU in MK cells.

Table 3. The Survival of Chick Embryos Upon Inoculation of Vaccinia

The CAM of one such infected but dead embryo was harvested, ground with sand and a 10% (w/v) suspension was prepared, and used for further inoculations. This resulted in higher rates of survival. For comparison with strains 117 and 118 only membranes from surviving embryos inoculated with the CAM-passed CV I were used.

The pocks were compared by visual observation rather than accurate measurements, supplemented with photographs. (Fig.4). In every instance, the pocks of CV I were smaller than the other two, which were of approximately equal sizes. They were compared both at 48 and at 72 hours with similar results. Moreover the pocks of 117 and 118, especially the latter, were elevated with a flat surface, opaque white, with a distinct margin and often showing umbilication with central
Figure 4. A. CV I, B. 117, C. 118, pocks on CAM at 48 hours.
(to be continued)
Figure 4. (continued) D. CV I, E. 117, F. 118, pocks on CAM at 72 hours.
ulceration and hemorrhage, more pronounced at 72 hours. The pocks of CV I were smaller, convex, pale white, with indistinct margins and showed no umbilication. With larger inocula, the embryos were dead and the membranes were thickened, hemorrhagic, shiny and friable, and no pocks were seen. In the case of 117 or 118, with larger inocula confluent pocks were seen even when the embryos were dead.

The histopathology of vaccinial pocks on CAM has been well described previously. (18,19). Briefly, endothelial and epithelial proliferation, cellular infiltration and hemorrhage with occasional epithelial ulceration are the main features. The epithelial and endothelial proliferation was more marked, the former often accompanied by ulceration, in the case of strains 117 and 118. Ulceration was also seen in CV I pocks in which the cellular infiltration tended to be more marked. Figure 5 shows a comparison of the histology of pocks of strains 117 and CV I. It was not difficult to differentiate CV I from the other strains by histology, but the differences were rather relative.

Discussion:

When Parker and co-workers studied CV I strain in comparison with others including strains CL and New York City Board of Health, they recorded that CV I killed chick
Figure 5. The pocks of CV I (A), and 117 (B) on CAM. (Magnification x30)
embryos more readily produced larger pocks than others and that large inocula resulted in thickened and shiny CAM, with no pocks. (6). The above results are in agreement with all but one finding, namely, the pocks are smaller than the other strains. There is no explanation for this discrepancy except that several passages had occurred in tissue culture when Parker studied CV I. It was shown in the previous chapter that CV I produces larger plaques in CEF.

Comparing the CV II strain with Elstree and Copenhagen—two European vaccine strains, van der Noordaa found smaller pocks being produced by CV II. He also found increased virulence of this strain for the chick embryo, and explained it in the light of faster dissemination of the virus in the embryo. (4).

Since CV I and II are closely related, embryonic chick cell-adapted strains with other similar properties, it is not surprising that they both are more virulent for chick embryos. It is, however, surprising that CV II and CV I produce smaller pocks on CAM, in spite of the fact that the latter produces larger plaques in CEF.

Summary:

The CV I strain was found to be more toxic or virulent for chick embryos. However, the pocks developed on the chorioallantoic membrane were smaller than those of strains
117 and 118. CV I pocks were pale white, convex, and with indistinct margins. The pocks of the other strains were opaque white, elevated, discoid, with distinct margins and often umbilicated. Morphologically, greater epithelial and endothelial proliferation was seen in them. On the other hand, a greater degree of cellular infiltration was seen in CV I pocks.
Neurovirulence in Young Mice

For the purpose of studying the sensitivity of the CV I strain to thiosemicarbazone, (Chapter 6) young mice were inoculated with this virus. Since all injected mice died within a short period, the neurovirulence of the three strains, 117, 118 and CV I were compared in young mice.

Materials and Methods:

Newborn or one week old Webster white mice were used for these studies. Each litter contained six or more sucklings, usually nine or ten.

In one experiment, approximately 100 PFU (in MK) of the virus strains were appropriately diluted in sterile 0.85% saline, and 0.01 mls. were injected intracerebrally in these mice. The daily mortality in these mice. Bacterial sterility of the inoculum was tested on blood agar plates.

The actual number of plaque forming units (PFU) injected in mice were determined in MK cells grown in tubes, according to the method described by van der Noordaa. (4). Briefly, the method consisted of draining off of the medium from MK tubes, washing the cells once with sterile saline if growth medium was on, inoculating actual mouse-doses or 0.1 mls. of appropriate dilutions, and adding medium after an adsorption time of one hour. Twenty four hours later, 0.1 ml. of
0.2% fowl erythrocytes in normal saline was added to each tube, incubated at room temperature for half hour and the hemadsorption-plaques were counted under low power magnification under a microscope.

Three mice each were collected at 24 and 48 hours after injection with the three strains of vaccinia. They were stored frozen until their brains were harvested and titrated. The brains were ground with sterile sand and three brains were suspended in 6 mls. of Hank's basic salt solution. Virus titrations were done in MK tubes.

Results:

In one experiment, approximately 100 PFU (in MK) of the three strains were injected in newborn mice. The daily mortality is shown in Figure 6. By the fourth day when all mice receiving CV I were dead, only 20% or less of mice receiving the other two strains had died. All of them were dead by the seventh day. In a second experiment in newborn mice, the inoculum was titrated in MK tubes to find the actual mouse doses as between 23 and 57 PFU. The daily mortality is shown in Figure 7. CV I and strain 117 behaved as in the previous experiment, but 118 caused a maximum of 90% mortality only by day nine.

Strains CV I and 117 were similarly injected in 7 day-old
Figure 6. Mortality of newborn mice, inoculated with strains CV I, 117 and 118.
Figure 7. Mortality of newborn mice inoculated with strains CV I, 117 and 118. Code same as in Figure 6.
mice. By simultaneous titration the mouse dose was found to be 13 PFU for both strains. As seen in Figure 8, CV I caused 100% mortality in 4 days, but a maximum of about 90% mortality was caused by 117, in 8 days.

Finally, brains in triplicate of newborn mice receiving 13 to 70 PFU of the three strains were assayed for virus content at 24 and 48 hours after inoculation. The results are shown in Table 4. The multiplication is expressed as the function of 1 PFU injected in the mouse.

<table>
<thead>
<tr>
<th>Strain</th>
<th>24 Hours</th>
<th>48 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>117</td>
<td>4.6</td>
<td>2.1 x 10^4</td>
</tr>
<tr>
<td>118</td>
<td>1</td>
<td>5 x 10^3</td>
</tr>
<tr>
<td>CV I</td>
<td>27</td>
<td>2.4 x 10^4</td>
</tr>
</tbody>
</table>

Table 4. The Multiplication of the Three Strains of Vaccinia in Mouse-brain, at 24 and 48 Hours

The fastest multiplication was shown by CV I and the slowest by strain 118.

Discussion:

Upon intracerebral inoculation, CV I caused death of young mice within four days. With low inocula, strain 118 caused a slower rate of mortality than 117, which in turn caused a slower rate than CV I. The rates of virus
multiplication in mouse brain was slowest for 118 and fastest for CV I. Therefore it seems likely that the faster mortality is caused by the faster multiplication of CV I in the brain.

Figure 8. Mortality of one week-old mice, inoculated with strains CV I and 117. Code same as in Figure 6.
multiplication in mouse brain was slowest for 118 and fastest for CV I. Therefore it seems likely that the faster mortality is caused by the faster multiplication of CV I in the brain.

It is rather surprising that a strain of virus adapted for growth in embryonic chick tissues appears to be more virulent for the brains of young mice. The significance of this finding, if any, is not known.

The CV II strain also exhibits greater neurovirulence in young mice compared with other strains. (20).

An incidental finding in the above studies is that the CV I strain has not lost the ability to cause hemadsorption by infected cells.

Summary:

Strain CV I was found to possess greater neurovirulence for young mice, than 117 or 118. This appeared to be due to the faster rate of multiplication of this strain in the brain.
Chapter 5

The Induction of, and Sensitivity to Interferon

Viruses vary in their ability to induce and cells vary in their ability to produce interferon. (21). Recently it was shown that attenuated strains of rubella and measles viruses are capable of inducing larger amounts of interferon than the parent or wild strains. (22, 23). In the light of these facts, the question arose as to whether the smaller plaques and slower rate of multiplication of CV I in MK cells were related to higher interferon production, in comparison to strains 117 and 118. Therefore, the following investigations were performed.

Materials and Methods:

Petri dishes of MK and CEF were prepared as described in Chapter 1. They were incubated for 24 hours with 2 mls. of medium containing 100 \( \mu \text{gm/ml.} \) of hydrocortisone (Solucortef, Upjohn). The media were removed and the cells inoculated with the three strains of vaccinia. The agar overlay also contained 100 \( \mu \text{gm/ml.} \) of hydrocortisone. Control dishes were treated as above, but without hydrocortisone. The plaque diameters were measured and the plaques enumerated as in Chapter 1.
Vesicular stomatitis virus (VSV) was obtained from Samuel Baron. It was passed once in MK cells. It was attempted to titrate VSV both by plaque assay as for vaccinia and by determining tissue culture infective dose (TCID<sub>50</sub>) in tubes of MK cells, according to the formula of Reed and Muench. (25). Since VSV plaques in secondary monkey kidney cells were stained red with neutral red, they were not easily recognisable.

Interferon was prepared by inoculating MK cells with VSV at a multiplicity of infection between 0.5 and 1 TCID<sub>50</sub>, and harvesting the fluids after 24 hours. For the induction of interferon by vaccinia virus, the three strains of viruses were inoculated in MK cells at a multiplicity of infection of approximately 0.1. The fluids were harvested 24 hours later. These interferon preparations were acidified to pH 2 using HCl, kept for a day at 4°C, brought back to pH 7 with NaOH, and kept frozen at -70°C.

All the interferon preparations were used at dilutions of 1/10 and 1/40, and aliquots of the former dilution were tested for the presence of residual virus in duplicate petri dishes and 5 MK tubes per interferon preparation. One ml. each of the interferon dilutions was laid over MK cells in petri dishes, incubated at 38°C. for 18 hours, drained off
and the cells inoculated with appropriate virus dilutions, for interferon assay.

Interferon was assayed by modification of a method suggested by Hermodsson and Philipson. (25). MK cells incubated for 18 hours with interferon dilutions were inoculated with VSV at a multiplicity of approximately 10, in 0.25 mls., rocked every half hour for two hours, and 1.75 mls. of maintenance medium added per petri dish. Twenty four hours later the supernatant alone was harvested and stored frozen. All such harvests were titrated in MK tubes to determine the yield as TCID$_{50}$. Control dishes were handled similarly, without treatment with interferon.

Results:

The number or the diameters of plaques produced in cells treated with hydrocortisone did not vary significantly from control plaques in untreated cells. This was true both in CEF and MK cells and for all three strains of viruses. The average variation in diameters determined from duplicate experiments in untreated and treated MK cells was approximately 10% or less for all three strains.

The preparations of interferon induced by the three vaccinia strains were free of infectious particles. However, one of five tubes inoculated with VSV induced interferon showed cytopathic effect. Therefore, when the latter was
assayed, the supernatents of 4 dishes incubated for 18 hours with VSV induced interferon were harvested and tested for infective particles in four MK tubes. No cytopathic effect appeared.

The yield of VSV from duplicate dishes incubated for 18 hours with various interferon preparations and challenged with VSV, are shown in Table 5. The yield is expressed as per cent of that obtained from control dishes not treated with interferon.

<table>
<thead>
<tr>
<th>Interferon Induced By</th>
<th>Reciprocal of Dilution</th>
<th>Yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>CV I</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>CV I</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>117</td>
<td>10</td>
<td>46</td>
</tr>
<tr>
<td>117</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>118</td>
<td>10</td>
<td>4.6</td>
</tr>
<tr>
<td>118</td>
<td>40</td>
<td>46</td>
</tr>
<tr>
<td>VSV</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>VSV</td>
<td>40</td>
<td>21</td>
</tr>
</tbody>
</table>

Table 5. The yield of VSV from MK cells treated with interferon induced by the three strains of vaccinia and VSV, and from untreated cells.

When 50% reduction in the yield of the challenge virus is taken as the measure of activity of one unit of interferon,
CV I, 117 and 118 induced approximately 20, 10 and 40 units each per ml. (25). VSV induced more than 40 units per ml.

To determine the sensitivity of the three strains of vaccinia to interferon, they were used as challenge viruses in MK cells incubated for 18 hours with five serial two-fold dilutions of the VSV-induced interferon starting from 1/10. Compared with control dishes, no plaque reduction occurred for any strain, even at 1/10 dilution of interferon.

Discussion:

Adrenocorticosteroids have been shown to inhibit interferon production both in cell cultures and in experimental animals. (26, 27). If interferon played a significant role in the development of small plaques by CV I, the pre-treatment of cells with hydrocortisone would have resulted in larger plaques. In two experiments in MK, once the plaques were slightly smaller and the second time slightly larger. The average variation was less than 7.5% and considered to be insignificant. The other two strains produced slightly smaller plaques upon hydrocortisone treatment, but the variations were again considered to be insignificant. The efficiency of plaquing measured by the number of plaques per inoculum also did not vary upon hydrocortisone treatment.

Since the purpose of these investigations was to determine whether larger amounts of interferon were induced by the CV I
strain, or whether it was more sensitive to the action of interferon, only comparative studies on crude preparations of interferons were performed. No such differences of significance were noted with crude preparations. Therefore, the need did not arise for the characterisation of the inhibitory substance as interferon, which it was presumed to be on circumstantial evidences.

Varying amounts of interferon were produced by the three strains of vaccinia. However, strain 118 produced more interferon than the other two strains. All three strains were relatively insensitive to plaque inhibition by interferon.

Summary:

Treatment of MK cells with hydrocortisone did not result in increasing the plaquing efficiency or plaque diameters of strains CV I, 117 and 118. They were relatively insensitive to plaque reduction by interferon induced in MK cells by vesicular stomatitis virus. Strain 118 induced more interferon than strain CV I which in turn induced more than strain 117.
Sensitivity to Thiosemicarbazone

N. methyl isatin B-thiosemicarbazone (NMBT) is an antiviral agent used in the prophylaxis of smallpox and the treatment of certain complications of vaccination. (28,29,30). Since CV I vaccinia is being used for human vaccination it was considered important to assess its sensitivity to this drug.

Materials and Methods:

NMBT was obtained as a 20% (w/v) suspension in 20% sucrose, from Burroughs Wellcome Laboratories.

The suspended drug was centrifuged at 2000 r.p.m. in an International Centrifuge, the sediment washed three times in sterile 0.85% saline, and a saline suspension prepared so as to contain an estimated 100 mg/ml.

Aliquots of the saline suspension were dissolved in warm 1N.NaOH, and appropriately diluted in doubly distilled water for use in tissue culture. The final solutions had a pH of 7 to 7.8. The solution was freshly prepared each time before use since it was found that the antiviral activity diminished on keeping if dissolved in NaOH. One such aliquot was dissolved in NaOH, extracted in acidified toluene and assayed for the drug content in a photoelectrometer, according to
the method described by Kempe et al. (31).

Three litters of seven-day-old mice were inoculated intracerebrally with approximately 50 PFU of CV I vaccinia diluted in sterile saline. One litter was injected intraperitoneally and the other subcutaneously, with a saline suspension of NMBT, two hours before and 20 hours after the inoculation. The dose was 0.5 mg. of the drug in .05 ml. volume. The mice were inspected daily and mortality recorded for 14 days after inoculation.

The sensitivity of strains 117, 118 and CV I were compared in tissue culture. Two experiments were done in MK cells, once using a virus inoculum of 60 to 150 PFU and the drug concentrations of 1.5, 1 and 0.5 μgm/ml. of agar overlay medium, and a second time with a virus inoculum of 100 to 230 PFU and 0.1, 0.05 and 0.01 μgm/ml. of the drug. A third experiment was done using a 2 μgm/ml. of NMBT. The drug, dissolved in NaOH and diluted in distilled water, was placed in less than 0.25 ml. volumes, over the solidified agar overlay. Controls were done without the drug. Similar studies were also done in CEF, using 200 to 300 PFU per petri dish.

In one experiment 60 x 15 mm. plastic petri dishes containing MK cell monolayers were inoculated in duplicate with the vaccinia strains, enough to cause confluent plaques (1500 to 3000 PFU). Over the agar overlay, a filter paper
disc (13 mm. diameter) was placed in the center, and was saturated with 10 μgm. of NMBT solution. Two days later the cells were stained with 0.01% neutral red, and the zones of plaque inhibition measured.

Results:

The solution of NMBT, estimated to contain 100 mg/ml, was assayed and found to contain 94 mg/ml. All the drug doses described are based on the assumption that the original suspension contained 100 mg/ml.

The results of the study in mice are shown in Figure 9. The challenge virus inoculum was sufficient to kill all mice in four days. However, two injections of NMBT reduced the mortality to 17% (drug given subcutaneously) and 33% (intra-peritoneally).

The percent plaque survival of the various strains in the presence of the drug in MK and CEF are shown in Figures 10 and 11. In MK and CEF, strains 117 and 118 showed approximately equal drug sensitivity. However, CV I showed a remarkable difference between the sensitivities in MK and CEF. In MK cells CV I was extremely susceptible to plaque inhibition by NMBT, whereas in CEF, its susceptibility was closer to that of strains 117 and 118.

Figure 12 exhibits the plaque reduction in MK cells by NMBT, of the three strains of vaccinia. 0.1 μg/ml of NMBT
Figure 9. The effect of NMBT on mortality of newborn mice inoculated with CV I. Solid circle represents untreated mice, solid triangles represent mice treated intraperitoneally and empty triangles represent mice treated subcutaneously.
Figure 10. The effect of NMBT on plaque formation by strains CV I, 117 and 118, studied in MK cells.
Figure 11. The effect of NMBT on plaque formation by strains CV I, 117 and 118, studied in CEF. Code same as in Figure 9.
Figure 12. The effect of NMBT on plaque formation by A. CV I, B. 117 and C. 118 at various drug levels.
was able to completely suppress plaque formation by CV I.

Progressively increasing doses of NMBT was observed to cause a reduction in plaque size also. The variation in average plaque diameters are shown in Table 6. It is measured as per cent reduction at each drug level.

<table>
<thead>
<tr>
<th>Dose of drug ug/ml</th>
<th>Variation in plaque size, % reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>117</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>.01</td>
<td>1.2</td>
</tr>
<tr>
<td>.05</td>
<td>20</td>
</tr>
<tr>
<td>0.1</td>
<td>26.5</td>
</tr>
<tr>
<td>0.5</td>
<td>42.7</td>
</tr>
<tr>
<td>1.0</td>
<td>46.6</td>
</tr>
<tr>
<td>1.5</td>
<td>53.7</td>
</tr>
</tbody>
</table>

-No plaques

Table 6. The progressive reduction in plaque size with increasing doses of NMBT.

The zones of plaque inhibition caused by NMBT in duplicate petri dishes inoculated with 1500 to 3000 PFU are presented in Table 7.

<table>
<thead>
<tr>
<th>No.</th>
<th>Diameters of zone of inhibition (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>117</td>
</tr>
<tr>
<td>1</td>
<td>41 x 42</td>
</tr>
<tr>
<td>2</td>
<td>40 x 40</td>
</tr>
</tbody>
</table>

Table 7. The diameters of the zones of plaque inhibition by NMBT.
Strains 117 and 118 had a peripheral zone of confluent plaques and a central zone of small, discrete and sparse plaques. The latter is designated as the zone of inhibition. CV I was prevented from causing any plaques; hence, the zone of inhibition was larger than the area of the petri dish.

Discussion:

The results presented in Figures 10 and 12 and Tables 6 and 7 indicate the markedly higher sensitivity of CV I in MK cells to NMBT, compared with the other two strains. Strains 117 and 118 were approximately of equal sensitivity. However, when the studies were performed in CEF, the pattern of drug sensitivity did not alter for strains 117 or 118, but CV I showed decreased sensitivity, approaching but not equalling that of the other two strains. (Figure 11). The reasons for this phenomenon are not known.

Thiosemicarbazone inhibits viral replication during the late phases of the intracellular events. (32). In MK cells it appeared as if the slower rate of replication made the CV I strain more susceptible to drug inhibition. However, strains 117 and 118 showed no significant differences in spite of their varied rates of multiplication. As suggested by the plaque sizes, all the three strains appear to multiply slower in CEF, but the drug sensitivities did not alter for strains 117 and 118. Again, assuming that CV I grows fastest
in CEF (since it produces the largest plaques), the drug sensitivity is not greater than that of the other strains.

The relatively small dose of 1 mg. per newborn mouse (approximately 0.03 mg/gm. body wt.) given subcutaneously was sufficient to protect about 83% from a 100% lethal dose of CV I. Given intraperitoneally, 67% were protected.

Thus, both in vitro and in vivo, the efficacy of NMBT against the CV I strain has been demonstrated. These may be taken as suggestive evidences for its potential use in man, should any untoward complications such as eczema vaccinatum or progressive vaccinia occur with CV I.

Summary:

The strong antiviral activity of N-methyl isatin B-thiosemicarbazon (NMBT) has been shown against the CV I strain, both in secondary monkey kidney (MK) cells and in newborn mice. In MK cells, this strain shows a greater sensitivity than strain 117 and 118, providing another marker for it. However, in chick embryo fibroblasts, the difference in sensitivity was not great.
Summary and Conclusions

In secondary rhesus monkey kidney (MK) and HEp 2 cells, CV I produced the smallest, strain 117 the intermediate and 118 the largest plaques. This order of plaque sizes was the same as the order of the rates of multiplication in MK cells, thereby suggesting that the rate of multiplication determined the plaque size. The plaque borders of 117 and 118 stained deeper with neutral red, but not those of CV I. In chick embryo fibroblasts (CEF) the order of plaque sizes were reversed, CV I producing the largest and 118 the smallest plaques. Compared with the plaquing efficiency in MK cells, that in CEF was 100% for CV I, but only 36 to 38% for 118 and 117.

The CV I strain was found to be more toxic or virulent for chick embryos. However the pocks developed on the chorioallantoic membrane were smaller than those of strains 117 and 118. CV I pocks were pale white, convex, and with indistinct margins. The pocks of the other strains were opaque white, elevated, discoid, with distinct margins and often umbilicated. Morphologically, greater epithelial and endothelial proliferation was seen in them. On the other hand, a greater degree of cellular infiltration was seen in CV I pocks.

Greater neurovirulence was exhibited by CV I for young
mice, than 117 or 118. This appeared to be due to the faster rate of multiplication of CV I in young mouse-brains.

Treatment of MK cells with hydrocortisone did not result in increasing the plaquing efficiency or plaque diameters of strains CV I, 117 and 118. They were relatively insensitive to plaque reduction by interferon induced in MK cells by vesicular stomatitis virus. Strain 118 induced more interferon than strain CV I which in turn induced more than strain 117. Thus, it appears that the differences in virulence, growth rate and plaque sizes are not related to the amount of interferon induced.

The strong antiviral activity of N-methyl isatin B-thiosemicarbazone (NMBT) has been shown against the CV I strain, both in secondary monkey kidney (MK) cells and in newborn mice. In MK cells, this strain shows a greater sensitivity than strain 117 and 118, providing another marker for it. However, in chick embryo fibroblasts, the difference in sensitivity was not great.

The serial passage of various viruses such as yellow fever, measles and vaccinia, in chick embryo tissues have resulted in the attenuation of virulence for man. Such attenuation, expressed as diminished plaque size and growth rate is also observed in tissue cultures foreign to the one in which serial passage has been conducted. This has been
shown with Edmunston strain of measles in primary and continuous line cells derived from African green monkey, with CV II in two human cell lines, and now with CV I in rhesus monkey kidney and HEP 2 cells. (4,14,15). These strains have not previously been studied in chick embryo tissue cultures. In the present investigation CV I appears to be more virulent in them according to the above criteria. Indeed, CV I and CV II are more virulent for chick embryos. (4,6). Thus, virulence and attenuation have to be considered with reference to adaptation. At least in the case of vaccinia, embryonic chick cell-adapted strains are more virulent for themselves and less virulent for cells of rabbits, monkey and man, in situ or in culture (4,6). By these same criteria, vaccinia adapted in mammalian tissues, such as strains 117 and 118 are less virulent, or attenuated for chick embryos and their cells. This may perhaps be true with other viruses also.

These are suggestive evidences, both in the case of measles and vaccinia, that the degree of attenuation is dependent on the passage levels in chick tissues. (6,16). Therefore, the change appears to be one of adaptation, and it can be predicted that as adaptation increases, virulence for foreign cells will further decrease. Parker studied a strain of vaccinia, passed serially well over 200 times in
chick embryo tissue culture, and it failed to replicate in rabbit testes. (6). It had lost all virulence for this tissue.

The attenuation resulting from serial passage in chick cells may not be universal for all foreign cells. Thus, CV I has been shown to be more neurovirulent for young mice than the other strains. The significance of this finding is unknown. It is also not known whether the WR strain which is adapted in mouse brain is more virulent for chick embryos.

In spite of being more virulent for the chick embryo, strains CV I and CV II produce smaller pocks on the CAM, than strains 117 and 118. Pock function is more complex than plaque formation and factors other than multiplication rates are likely to be involved in this process. However, after well over 200 passages in chick cell cultures, Parker found that the CV I strain produced larger pocks. (6).

Evidence suggests the increased induction of interferon by attenuated strains of rubella and measles viruses over the unattenuated strains. (22,23). The present investigations, though limited, do not suggest such a property for the CV I strain. All three strains of vaccinia were rather insensitive to interferon.

For certain live virus vaccines, it is important to have
markers for the identification of the attenuated strains. This is particularly true of attenuated polio viruses. For CV I, the detection of markers are less important. However, for further studies into the basic mechanisms of adaptation and modification of virulence, markers may be required. Several such markers are suggested by the above investigations. Pock and plaque sizes are relative in any experiment; so also are the growth rates and virulence for chick embryo and young mice. The sensitivity to thiosemicarbazone seems to be the most efficient marker. In the presence of NMBT, at levels of 0.1 or more μgm/ml of the medium, in MK cells, no plaques are produced by CV I. The other two strains are completely suppressed only at levels above 2 μg/ml.
Appendix

Recipes for media used in the reported investigation:

Growth medium for monkey kidney cells:

Minimum essential medium in Earle's BSS* 88 mls.
Bovine serum** 10 mls.
Antibiotics*** 1 ml.
Glutamine 1 ml.

Maintenance medium for monkey kidney cells:

Lactalbumin Hydrolysate 0.5 gm.
Fetal calf serum** 2 mls.
Hank's BSS* 97 mls.
Antibiotics*** 1 ml.

Growth medium for HEp 2 cells:

Eagle's basal medium in Earle's BSS* 88 mls.
Bovine serum** 10 mls.
Antibiotics*** 1 ml.
Glutamine 1 ml.

Maintenance medium for HEp 2 cells:

Eagle's basal medium in Earle's BSS* 96 mls.
Fetal calf serum** 2 mls.
Antibiotics*** 1 ml.
Glutamine 1 ml.

Growth medium for chick embryo fibroblasts:

Medium 199* 89 mls.
Antibiotics*** 1 ml.
Bovine serum** 10 ml.
Maintenance medium for chick embryo fibroblasts:

Medium 199*

Antibiotics**

99 mls.

1 ml.

*including NaHCO₃ for final pH of 7.2

**heat inactivated

***penicillin 250,000 units, streptomycin and neomycin

25,000 µg each and bacitracin 25 units/100 mls.
References


