

129

AN INVESTIGATION OF THE MANIFESTATIONS OF VITAMIN E DEFICIENCY
AND THEIR POTENTIAL RELATIONSHIP TO CHLOASMA GRAVIDARUM

by

(RUTH) ELIZABETH SPICHER

A technical paper submitted to the Graduate Faculty in
partial fulfillment of the requirements for the degree

of

MASTER OF NURSING

Approved:

Mrs Laura Walker RN PhD
Head, Major Department

Phyllis Hillard
Chairman, Examining Committee

F Goering
Dean, Graduate Division

MONTANA STATE UNIVERSITY
Bozeman, Montana

June, 1969

ACKNOWLEDGMENT

The investigation of this phenomenon would not have been possible except for the cooperation of many persons who deserve recognition.

The acceptance of an unusual research design was permitted by the members of the graduate committee, Mrs. Barbara Hauf (Chairman for the first year), Miss Rita Darragh, Mrs. Lucille Wilson, and Miss Bethine Bigej. Mrs. Phyllis Hillard assumed the responsibility of Chairman when Mrs. Hauf left for graduate study. Both Chairmen encouraged and guided this investigator through the various developing stages of this study and exercised much patience. Mrs. Hauf was especially generous as she guided the beginning efforts of research.

Because the research of this phenomenon led into biochemistry and this researcher had a limited background in this area, Dr. David G. Stuart, Dr. William Hill, and Dr. Samuel J. Rogers of the Montana State University faculty gave needed counsel and read portions of the paper to detect possible errors.

Dr. Albert Suvak guided the statistical analysis and interpretation of the data in Chapter VII.

Mrs. Lois Johnson typed the manuscript--giving attention to much detail.

Thanks is due to my husband and family who tolerated the interruptions of family life that long hours of study required to complete this study.

TABLE OF CONTENTS

PART I: THE PROBLEM SITUATION

CHAPTER	PAGE
I. INTRODUCTION	2
A. Phenomena	2
B. Investigator's Observations	3
C. The Statement of the Problem	4
D. The Purposes of This Study Are	5
E. Definitions	5
F. Organization of This Study	6
II. METHODOLOGY	7
A. The Choice of a Research Method	7
B. The Phases of Open-Ended Research	9
C. Methodology of the Study of Postpartum Patients With Chloasma Gravidarum	10
D. Limitations of This Study	11
E. Assumptions	12

PART II: THE LITERATURE SURVEY

III. VITAMIN E AND ITS DEFICIENCY	15
A. Historical Perspective	15
B. Definition of Vitamin E	15
C. The Functions of Vitamin E	16
D. Manifestations of Vitamin E Deficiency in Animals	21
E. Vitamin E Deficiency in Humans	26

CHAPTER	PAGE
IV. PIGMENTATION	32
A. Facts Concerning Pigmentation	32
B. Tissue Pigmentation and Lipid Oxidation	37
C. Pigmentation in Various Tissues	42
D. The Time Factor	43
E. Pathological Conditions with Pigmentation as a Manifestation	43
F. A Summary of the Findings Relative to Pigmentation in Literature	48
V. VITAMIN E DEFICIENCY, INCREASED MELANIN PRO- DUCTION AND REPRODUCTION	51
A. The Effect of Vitamin E Deficiency on Reproduction	51
B. The Effect of Vitamin E Deficiency on the Fetus and on Infants	54
C. Caplan's Observation of Schizoid-Like Behavior in Pregnant Women	56
D. Summary	59
VI. A BROAD VIEW OF VITAMIN E DEFICIENCY AND PIGMENTATION	60
A. A Summarization of the Functions of Vitamin E	60
B. The Emerging Clinical Picture of Vitamin E Deficiency	60
C. Consideration of Requirements of Tocopherol	63
D. The Nutritional Availability of Vitamin E	65

CHAPTER	PAGE
VI. E. Varied Uses of Tocopherol	67
F. A Summary of the Literature Survey	67
PART III: A STUDY OF POSTPARTUM PATIENTS WITH CHLOASMA GRAVIDARUM	
VII. THE ANALYSIS OF DATA COLLECTED ON POSTPARTUM PATIENTS WITH CHLOASMA GRAVIDARUM (AND THEIR INFANTS)	72
A. Review of Methodology of This Specific Study	72
B. Results of This Study	73
C. Summary and Conclusions of This Study of Patients With Chloasma	82
PART IV: THE RESULTS	
VIII. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS	85
A. Summarization of the Segments of This Study	85
B. Discussion and Recommendations Regarding Study of Postpartum Patients	87
C. Repetitious Factors	90
D. Hypotheses For Future Study	93
E. Tools to be Developed and Validated	96
F. Implications for Nurses and Other Health Personnel	97
G. Conclusion	100

CHAPTER	PAGE
APPENDIX A:	102
Information Collection Form	103
Rating Scales	104
APPENDIX B:	105
The Normal Cell	106
LITERATURE CITED	117

LIST OF TABLES

TABLE		PAGE
I.	Classification of Postpartal Patients With And Without Chloasma	74
II.	A Comparison of Age and the Presence or Absence of Chloasma Gravidarum in Postpartum Patients While Hospitalized	75
III.	A Comparison of Gravida and the Presence or Absence of Chloasma Gravidarum in Postpartum Patients While Hospitalized	76
IV.	A Comparison of Hematocrit and the Presence or Absence of Chloasma Gravidarum in Postpartum Patients While Hospitalized	77
V.	A Comparison of Infant Weight (As An Index of Prematurity) and the Presence or Absence of Chloasma Gravidarum in Postpartum Patients While Hospitalized	78
VI.	A Comparison of Due Date and the Presence or Absence of Chloasma Gravidarum in Postpartum Patients While Hospitalized	79
VII.	A Comparison of Miscarriage Rate and the Presence or Absence of Chloasma Gravidarum in Postpartum Patients While Hospitalized	80

TABLE	PAGE
VIII. A Comparison of Postpartal Flow and the Presence or Absence of Chloasma Gravidarum in Postpartum Patients While Hospitalized	81
IX. A Comparison of Ketones in Urine and the Presence or Absence of Chloasma Gravidarum in Postpartum Patients While Hospitalized	82

LIST OF FIGURES

FIGURE		PAGE
1.	Metabolic Pathways of Phenylalanine and Tyrosine	58

ABSTRACT

The problem of this study is to determine if there is any relationship between chloasma gravidarum and vitamin E deficiency. The three aspects include: 1) what factors are known about vitamin E; 2) the clinical picture of vitamin E deficiency; and 3) chloasma gravidarum and the symptoms exhibited by patients with this phenomenon.

Open-ended research, that allowed more freedom to explore, aided in the evolution of hypotheses that were researched as an outgrowth of the findings in the literature review. The method examines similarities and differences to identify hypotheses for research.

The purposes of the study were: 1) to accumulate and classify data, and to show possible relationships or the absence of relationships; 2) to generate hypotheses relative to the main segments of this study--vitamin E deficiency and chloasma gravidarum; and 3) to identify and indicate possible implications for nursing.

A common factor that seemed to be present in each facet of the study seemed to involve fat metabolism. 1) vitamin E is fat soluble and its deficiency is associated with steatorrhea. 2) melanin stimulating hormone, that regulates melanin production, may also be involved in regulating fat metabolism. 3) and, ketones in the urine are related to increased fat metabolism. The study includes a description of the functions of vitamin E and the pathology associated with its deficiency as well as a description of the emerging clinical picture. An exploration of the little known about chloasma gravidarum led to the study of forty-six postpartum patients in a hospital setting. Of these patients, fifteen had some degree of chloasma and thirty-one did not. In addition it revealed that women who have chloasma are inclined: 1) to be older; 2) to be multiparas; 3) to have a heavier postpartal flow; 4) to have ketones in their urine; and 5) though their infants are born a little earlier, it is not at a significant level.

Over forty hypotheses were formed for further research. A tool used to evaluate chloasma gravidarum needs further validation and possible revision, as it may be useful in helping nurses to assess and record the degree of chloasma gravidarum on charts so that research can be done relative to this phenomenon. An implication of importance is the need for nurses, when planning health care, to consider the fact, that vitamin E is essential in a right ratio relationship to polyunsaturated fatty acids in human nutrition. More definitive research may possibly reveal a relationship between chloasma gravidarum and vitamin E deficiency though this could not be established by this research.

PART I

THE PROBLEM SITUATION

CHAPTER I

INTRODUCTION

A. Phenomena

Time and again it has been shown that out of unexplained phenomena can come new knowledge. A phenomenon is "that which is open to observation; an event; a fact."¹ e.g., Hans Selye observed the phenomenon of "generalized illness and that all persons, regardless of their diagnosis, presented a set of common symptoms." Years later he was able to relate this to the result of unexplained changes of the thymus gland, stomach, and the adrenal glands of experimental rats; and thus the "general adaptation syndrome" eventually became recognized and influenced the whole of medicine and nursing. "It is not to see something first, but to establish a solid connection between the previously known and the unknown that constitutes the essence of scientific discovery."²

The "mask of pregnancy" is a phenomenon that has been considered insignificant for years. It has been observed but considered so inconsequential that it has not even been recorded on patients' charts. Doctors tell their patients not to be concerned about the condition as the blotchy discoloration will go away following pregnancy. It often does disappear, but for a few persons it remains, at least to some degree. In the opinion of this investigator the "mask of

¹Faye G. Abdellah, and Eugene Levine, Better Patient Care Through Nursing Research (New York: Collier-Macmillan Limited, 1965), p. 706.

²Hans Selye, The Adaptation Syndrome (Palo Alto, California: J. M. Stacey, 1952), Preface.

pregnancy" (chloasma gravidarum) is probably a sign of disease or deficiency, and she believes that if studies were done in an effort to relate it to the unknown, it could become a valuable symptom that would aid in identifying the disease or deficiency to which it might be related.

B. Investigator's Observations

The observation of a family group in which the mother and her six daughters all had the "mask of pregnancy" (chloasma gravidarum) is responsible for the initiation of this study. In this particular group the chloasma disappeared for most of them following pregnancy, but for two of the daughters a marked discoloration was retained following their last pregnancies. All but one of these daughters had miscarriages.

Of the two who retained chloasma, one tolerated a low level of health for approximately five years and was then finally diagnosed as having an arrested case of Addison's disease. Cortisone relieved many symptoms but when her youngest child was twelve years old, she had a complete break with reality. The son born to her at a time when her health was at a low ebb has always been hyperactive and is receiving psychiatric treatment.

The other daughter sought aid for exhaustion and an inability to regain her health. Nothing seemed to help. Her beautician recommended vitamin E to clear up the brown blotches on her face. She took some vitamin E to her doctor and offered to be a "guinea pig" for experiment. The doctor updated his knowledge concerning vitamin E and permitted her to take the vitamin E along with a balanced dosage of other vitamins. Within six months she was carrying out full responsibilities in her family; the brown blotches were still visible but not nearly

so prominent. Several years later she decided she no longer needed vitamin E and discontinued taking it. After three months the brown markings became more prominent and she had less energy. Her menstrual periods became irregular; in fact, they had almost ceased (she was 37 years old at that time). She resumed her previous dosage of vitamin E and has been taking it regularly since that time. She also adheres to a balanced diet as well as balanced dosage of other vitamins. Now, at 44 years of age, her menstrual cycle recurs every 26 days and is normal in amount.

The family history includes heart disease on the paternal side and diabetes, hay fever, asthma and eczema on the maternal side. These diseases have reappeared in the next generation, as well. One brother, to the six sisters mentioned above, died of coronary occlusion at 37 years of age. His skin, from childhood on, was very dark and during adolescence it had a blotchy appearance.

The introduction to this specific family group that excited the investigator's interest is included here to aid the reader in seeing some of the factors that could be included in this multifaceted problem. Many of the diseases that occurred in this family group have been known to occur relative to vitamin E deficiency and to respond to treatment with vitamin E.

C. The Statement of the Problem

Do women who have chloasma gravidarum (and their infants) have or develop one or more of the various symptoms or conditions of vitamin E deficiency as it is depicted in the literature?

1. What factors are known about vitamin E--its functions and its relationship--if any, to pathology?

2. What is the clinical picture of vitamin E deficiency?
3. What is known concerning chloasma gravidarum? What symptoms do patients manifest who exhibit this phenomenon?

D. The Purposes of This Study Are:

1. To accumulate and classify data regarding chloasma and vitamin E deficiency; and to show possible relationships or the absence of relationships.
2. To generate hypotheses relative to vitamin E deficiency and chloasma gravidarum.
3. To identify and indicate possible implications for nursing.

E. Definitions

The many technical terms included in this paper are defined within the context of the paragraph in which it is used. Two terms need to be defined here for the purposes of this paper.

Chloasma gravidarum, an irregular yellow-brown pigmentation of the face, is most commonly known as "the mask of pregnancy." Dorland's medical dictionary states that it is "a discoloration of the skin occurring in pregnancy, with brownish areas usually on the upper part of the face, forehead and cheeks; melasma gravidarum; a discoloration of the skin of pregnant women."³ Fitzpatrick, et al., define it more clearly as "irregular spots or blotches of muddy

³ _____, Dorland's Illustrated Medical Dictionary, 24th ed. (Philadelphia, London: W. B. Saunders Company, 1965), pp. 291-292 and 891.

brown color (that) appear on the face"⁴ during pregnancy. It is more prominent in some persons than in others.

Tocopherol is the technical name for vitamin E. Seven different forms have been identified. Most of the research related to tocopherol deficiency is related to alpha-tocopherol; therefore, for the purposes of this paper, tocopherol will refer to a-tocopherol except when specified as one of the other tocopherols.

F. Organization of This Study

This study involves two main parts. In Part II the literature was searched to find what is known about vitamin E deficiency, chloasma and pigmentation. Part III involved an actual study of patients with chloasma gravidarum.

⁴Elise Fitzpatrick, Nicholson J. Eastman, and Sharon R. Reader, Maternity Nursing, 11th ed. (Philadelphia, Toronto: J. B. Lippincott Company, 1966), p. 117.

CHAPTER II

METHODOLOGY

A. The Choice of a Research Method

Beginning with observed phenomena, the type of research methodology for this study needed to be flexible--a method that would allow "free and unhampered inquiry after truth."⁵ In the introduction of Explorations in Communications, the authors state:

If you beam knowledge at a situation, you find it is quite opaque: if you organize your ignorance, tackling the situation as an over all project, probing all aspects at the same time, you find unexpected aperatures, vistas and breakthroughs. Thus the chemist Mendeleev, to discover the missing group in the element chart, did not simply use available knowledge. Instead he asked, "what must be the characteristics of the rest, if those we know are to make sense among themselves?"...light through, rather than light on... (that is) total illumination from within (in contrast to) perception and analysis that deliberately, and by organized means ignores all but one thing at a time... This intensity of analysis is achieved at the price of forcing all else in the field of perception into the subliminal... fragmenting of the field of perception and breaking of movement into static bits, man has achieved a power of applied knowledge and technology unrivaled in human history. The price we pay is existing personally and socially in a state of almost subliminal awareness.⁶

Because of their overlapping functions, nurses have the opportunity for thoughtful participation in the observation of specific phenomenon and unexplained symptoms. The basic observation plus a search for other phenomena or facts, that may or may not be interrelated, can be productive of evidence that may link isolated segments of researched material. This integration of facts may add to

⁵Tyrus Hilway, Introduction to Research, 2nd ed. (Boston: Houghton Mifflin Company, 1964), p. 53.

⁶Edmund Carpenter, and Marshall McLuhan, Explorations in Communications (Boston: Beacon Press, 1960), paperback edition, 1966, pp. X-XI.

basic knowledge and in this way affect the practice of all of the health professions.

Fragmentation is the necessary evil that accompanies the specialization that is so essential amidst the mushrooming of new information. Every fragment of new knowledge seems to generate more specialized answers--answers that can come only through progressive specialization. This type of research must continue if there is to be progress.

However, integration is essential to gain perspective. It seems that the nurse who works with the whole person is in the unique position to see the relatedness of certain phenomena.

Chloasma is an example of a phenomenon which is generally not seen as related to various other phenomena or symptoms related to pregnancy. Its commonness and seeming insignificance make it unattractive to the researcher. However, it may be a connecting link to related information, and so, it can only be studied in relation to the whole or in a study setting that promotes perspective as well as specialization.

The usual methods of formulating an hypothesis and testing it is inadequate for this study. Because of this, the investigator chose to use open-ended research.

Open-Ended Research. Open-ended research indicates a method of investigation in which theories and hypotheses are derived as an out-growth of the material and data studied without preconceived hypotheses to be tested. It seeks out facts, phenomena, and relationships.

Through a process of cyclic progression, it sifts or refines the data, definitions are formed, hypotheses are generated, findings and data sifted, and deductions made to form theories for further testing. Inferences can be based

on researched experiments found in literature. Continued refinement by this process eventually evolves theories and postulates.^{7, 8}

B. The Phases of Open-Ended Research as used by the investigator for this study were:

Part I The Observation of a Phenomena

Part II Literature Survey

1. An attempt to find order in the readings;
2. Hunches and hypotheses were derived from readings to see how they might fit;
3. Many were eliminated;
4. Others emerged to form significant categories for this study;
5. Specific aspects of the field were studied
 - a. the clinical picture of vitamin E deficiency
 - b. pigmentation
 - c. pregnancy and reproduction;
6. The identification of similarities and differences of opinions, and findings of researchers;
7. The pinpointing of specific types of information to be gained for further study.

Part III A Study of Postpartum Patients with Chloasma Gravidarum

1. A sample of immediate postpartum mothers with their infants was selected;
2. Observations were made and data collected;
3. The analysis of the data; collections and analysis become more specific;
4. An attempt at pinpointing relevant hypotheses.

Part IV The Results

1. Findings of this study;
2. The pinpointing of hypotheses for investigations in future studies;
3. Statement of Implications for Nurses.

⁷Jeanne C. Quint, "The Case for Theories Generated from Empirical Data," Nursing Research, Spring, 1967, pp. 109-114.

⁸Abdellah and Levine, op. cit., pp. 69-71.

C. Methodology of the Study of Postpartum Patients with Chloasma Gravidarum

Sample. For purposes of this study, the cohort method of delineating a sample was chosen.⁹ "The mask of pregnancy" was used as the identifying characteristic to differentiate between two groups of subjects. Group I included fifteen patients who had chloasma to some degree; and Group II included thirty-one patients who did not have chloasma. After the degree of chloasma gravidarum was rated on postpartum patients, information was first collected on the group with chloasma. When certain types of information seemed to be repetitious, the same kinds of information were collected from the charts of women without chloasma so that this group could be used as a control group of subjects.

Data Collection. The temperature taking procedure was used as a method of observing postpartum patients in a hospital setting. This method of rating the degree of their "masks" was chosen to prevent any anxiety that undue concern for their masks might produce.

Tools. Three tools were developed for the purposes of this study (Appendix A). A rating scale was developed for the purpose of assigning a degree of chloasma gravidarum present in postpartum patients. Another rating scale was developed to evaluate the amount of postpartal flow.

The Information Collection Form was the third tool used. This form was tested during the investigator's first week of clinical experience in the

⁹Ibid. (The cohort method of delineating a sample uses an identifying characteristic and explores retrospectively for the potential dependent variable or variables. It is the independent variable in a longitudinal study. Cohort studies are essentially cross-sectional studies involving the same subjects. There are usually so many variables present that it would be difficult to establish a causal relationship, but its value is in suggesting fruitful avenues of approach for future research.)

Billings Deaconess Hospital.

D. Limitations of This Study

The inability of the investigator to read thousands of validated studies relevant to vitamin E research is, in part, overcome by the availability of an excellent review by M. K. Horwitt and the published reports of a nutrition symposium that evaluated current knowledge of vitamin E and its deficiency.

The large number of interrelated variables make limitation of the scope of the problem difficult without losing the perspective desirable for identifying relationships. The scope of this study was limited, to some degree, by the use of the cohort characteristic (the mask of pregnancy) and by focusing on the repeated findings related to vitamin E deficiency.

Since many people are sensitive about having chloasma, some of the respondents might have become alarmed if questioned concerning it; therefore, it was necessary to avoid drawing attention to the "mask of pregnancy" as a significant sign or symptom. This necessity placed a limit on the possible approaches that could be used to gain information about the various aspects of the problem.

Comparisons of all possible aspects could not always be made because the kinds of information available on the charts of patients identified as having chloasma differed from chart to chart. This limitation was partially overcome by extracting and recording only the similar kinds of information that was available on charts. This meant, of course, that much information that might have been significant had to be disregarded.

The identified symptoms of diseases in the sample population may have

been related to other causes than to a vitamin E deficiency, as no attempt was made to control the variables. Interpretations need to be made cautiously and additional research is necessary to verify possible conclusions. Caution in interpretation has also been necessary for dealing with the possibility of bias on the part of the investigator.

Much of the literature is of a technical nature. The lack of biochemical background of the investigator made interpretation of the literature difficult. However, several books have been valuable in understanding the technical aspects of the study. In addition, several faculty members at Montana State University have helped the investigator gain understanding of some of the technical material and have also read parts of the paper to identify possible errors.

E. Assumptions

1. Chloasma does occur and is identifiable in some pregnant women.
2. "The skin changes (of pregnancy) may be associated with hypertrophy of the adrenal cortex of the adrenals."¹⁰
3. Tocopherol (vitamin E) deficiency does exist, can be produced, and is manifested in experimental animals in many ways.
4. Tocopherol (vitamin E) deficiency does exist for some humans and a vast amount of research indicates multiple and subtle manifestations of the disease.

¹⁰Fitzpatrick, op. cit., p. 118.

PART II

THE LITERATURE SURVEY

In order to understand the possible interrelationships of chloasma gravidarum and the multifaceted dimensions of vitamin E deficiency, as it relates to the pregnant woman and her infant, it is necessary to consider the cell and its many processes. The interrelationships of enzymes, hormones and vitamins, because of their interdependency, need to be understood.

Metabolic processes occur in the cell, the basic unit of life. Enzymes trigger these processes. Specialized cells secrete specialized macromolecules-- hormones--to perform specialized tasks. The cell can function only if it is provided with essential elements and nutrients. Vitamins are chemicals essential for the production of enzymes.

The interdependence of vitamins, enzymes and hormones, as a part of the body, are becoming better understood. Cooley states that "...vitamins are so many faceted, so interdependent, so interlinked with hormones, enzymes and other molecules, that their effects are manifested in every function of the body."¹

The literature survey reveals an interrelationship concerning 1) malnutrition at the biochemical level, 2) the aging of the human organism, and 3) stress as defined by Selye. A discussion of the above concepts is included in Appendix B, to which the reader will be referred to at various places in the review.

¹Donald G. Cooley, "What is a Vitamin," Today's Health, 41:73, June, 1963.

In Part II answers are sought to questions like: How does vitamin E function? What is the clinical picture of vitamin E deficiency as depicted in literature? What manifestations are related to vitamin E deficiency in animals and in humans? Is there a possibility that chloasma gravidarum is a significant sign or symptom of deficiency or disease? Could it be a symptom of the peroxidation of cellular lipoproteins that occurs when vitamin E is not present in adequate amounts? What are the similarities and differences of vitamin E deficiency as found by researchers? What are the similarities and differences in diseases that have pigmentation as a symptom? Since pregnancy is considered a stressor period in the life cycle of a woman, is her vitamin E reserve used up more rapidly and at times becomes depleted? Why is chloasma seemingly more prevalent in certain family groups? Does vitamin E deficiency affect the fetus during the prenatal period?

In this summary of a sampling of the literature, specific attention is directed to:

- 1) Vitamin E and its Deficiency (Chapter III);
- 2) Pigmentation (Chapter IV);
- 3) Vitamin E Deficiency, Increased Melanin Production and Reproduction (Chapter V); and
- 4) A Broad View of Vitamin E Deficiency and Pigmentation (Chapter VI).

CHAPTER III

VITAMIN E AND ITS DEFICIENCY

A. Historical Perspective

Vitamin E research is not new. The initial research was done about forty-five years ago by Evans and associates. They reported the discovery of a new substance that affected the fertility of the rat and named it vitamin E. In these early experiments the fact that there was a relationship between vitamin E and the type of fat intake was observed.² This vitamin is still known as the fertility vitamin.

In 1961 M. K. Horwitt--the former director of the Biochemical Research Laboratories at Elgin State Hospital, and later an Associate Professor of Biological Chemistry at the University of Illinois College of Medicine--reviewed the published papers on vitamin E. He limited himself to three percent of the papers that had been published up to that time and apologized for not being able to give attention to some very excellent contributions. His reference list included one-hundred and six studies.³ Since that time many more studies have been published. Chemistry Abstracts lists studies almost weekly on some phase of the complex and controversial subject of vitamin E deficiency. Nutritional journals are giving more space to research on vitamin E. Inconsistent reports of

²Herbert McLean Evans, "The Relations Between Fertility and Nutrition," Lectures on Nutrition, Mayo Foundation Lectures (Philadelphia: W. B. Saunders Company, 1924-1925), p. 219.

³M. K. Horwitt, 'Vitamin E in Human Nutrition--An Interpretative Review,' Borden's Review of Nutrition Research, 22:1:13, January-March, 1961.

authentic research, as well as unjustified claims of "food faddists," have added to the confusion relative to the already complex nature of vitamin E deficiency.

In this section of the literature review, specific attention is directed to (1) a definition of vitamin E, (2) studies that aid in delineating its functions, (3) studies of vitamin E deficiency in animals, and (4) studies of vitamin E deficiency in humans.

B. Definition of Vitamin E

Vitamin E is a fat soluble nutrient of which there are seven known forms of tocopherol.⁴ Alpha-tocopherol is the most common form and apparently the most essential form. Much of the research that has been done has been relative to a-tocopherol and, unless another tocopherol is designated, the use of the word tocopherol in this paper will refer to a-tocopherol. The tocopherols are heavy molecular alcohols and "they differ in the number and position of the ethyl groups, and differ somewhat in potency."⁵

C. The Functions of Vitamin E

Antisterility and Progestational Function. The antisterility and pro-gestational functions of vitamin E were recognized in the early 1920's by Evans, et al., for rats. This function has been verified and recognized by authorities

⁴C. Burke, "A Common Solvent Impurity Resembling a-tocopherol," J. Chromatogr. (Univ. York, England), 31(1), 247-250, 1967, as cited in Chemical Abstracts, Vol. 68, #10089d. (In addition to the seven known tocopherols, "A common solvent impurity was finally isolated and resembles a tocopherol... (from) bean leaves...")

⁵Cyril Keele, and Eric Neil, with collaboration of John B. Jebson, "The Vitamins," Samson Wright's Applied Physiology, 10th edition. (New York, London, Toronto: Oxford University Press, 1961), p. 527.

for a long time in animals, but it has not been considered essential for humans.⁶

Antianemic Function. Shortened red cell survival in vitamin E deficient monkeys has been demonstrated repeatedly. Oski and Barness demonstrated the antianemic function of tocopherol in premature infants at the hospital connected with the University of Pennsylvania. These infants had a hemolytic anemia and their serum tocopherol was low. Oski also noted other clinical signs in these infants. "Signs of hemolysis decreased after the administration of vitamin E."⁷ Binder, et al., indicated that there is a shortened red cell survival in vitamin E deficient adults with malabsorption syndromes. (Page 26) In these same patients "heinz bodies could be demonstrated in the absence of a specific enzyme."⁸

The Function of Development and Maintenance of Muscle Integrity.

Much research has been done relative to the development and maintenance of muscle integrity. White muscle disease in calves is attributed to tocopherol and selenium deficiency.⁹ Selenium is a trace mineral that has antioxidant properties. Harris states that in the rabbit, muscular dystrophy is the first manifestation of vitamin E deficiency. Smooth muscles, as well as striated muscles, are involved. This is indicated by gizzard erosion in the turkey and "sudden fatal

⁶Keele, et al., loc. cit.

⁷Frank A. Oski, and Lewis A. Barness, "Vitamin E Deficiency: A Previously Unrecognized Cause of Hemolytic Anemia in the Premature Infant," The Journal of Pediatrics, 70:2:219, February, 1967.

⁸H. J. Binder, D. C. Herting, V. Hurst, Finch, and H. M. Spiro, "Tocopherol Deficiency in Man," New England J. Med., 275:1289:597, 1965.

⁹Don Graham, "Selenium, Tricky Trace Element," Now, Montana State University, College of Agriculture, Bozeman, Montana, Fall, 1967, pp. 3-6.

failure of the heart muscle" in the cow. Harris also indicates that ceroid deposition--an insoluble pigment found in the fibrous tissue of cirrhotic livers--in muscle can be identified by biopsy. Creatinuria in humans with vitamin E deficiency indicates muscle breakdown. It is possible to produce muscular dystrophy in laboratory animals by depriving them of tocopherol and increasing the polyunsaturated fat intake.¹⁰

Antioxidant and Free Radical Scavenger Function. "There is nothing new in the idea that tocopherol acts as an antioxidant or that the addition of pro-oxidants (and/or) easily oxidized lipids will promote tocopherol deficiency.¹¹ Vitamin E acts as a cellular antioxidant by counteracting excessive oxidation of the lipoproteins from which the cell membranes or organelles are composed. The presence of tocopherol can counteract the red cell hemolysis that is associated with vitamin E deficiency.¹² According to Tappel's theory, the presence of vitamin E--a normal cell constituent--in adequate amounts can slow the aging process of the cells at the molecular level and thus the whole body is influenced (Appendix B). Selye's research supports this idea. Tappel's theory is given support when Horwitt cites the authority of E. Hove to state, "that if vitamin E truly acts as an in vivo antioxidant, the destruction of vitamin E during lipid peroxidation is a normal pathway and function, and not an artifact."¹³

¹⁰Phillip L. Harris, "Discussion" in a Symposium: Advances in the Detection of Nutrition Deficiencies in Man, The American Journal of Clinical Nutrition, 20:6:602-604, January, 1967.

¹¹Horwitt, op. cit., p. 1.

¹²Oski and Barnes, op. cit., pp. 211-220.

¹³Horwitt, op. cit., p. 5.

Tappel classifies antioxidants into three major categories:

1. Lipid antioxidants--vitamin E and selenium, a trace mineral.
2. Water-soluble antioxidants--glutathione, cysteine and sulfa-hydryl proteins.
3. Free radical scavengers--vitamin C, vitamin E, selenium, glutathione, cysteine and sulfahydryl proteins.¹⁴

Acting as a Repressor for Catabolic Enzymes. Olson states that the antioxidant powers of vitamin E "cannot of themselves, account for all of the manifestations of vitamin E deficiency." He proposes that vitamin E acts as a repressor for catabolic enzymes, many of which are found in the lysosomes--the garbage disposal unit of the cell.¹⁵

Enzyme repression is the process in which the rate of enzyme synthesis is decreased relative to the normal rate,

as opposed to enzyme induction--

the process which increases the rate of synthesis of a specific enzyme relative to its rate of synthesis in the uninduced organism.¹⁶

Olson provides evidence for his hypothesis:

...that a-tocopherol has a repressor function in cooperation with a regulatory gene to control the excessive synthesis of catabolic and redundant enzymes. This can be reconciled with the hereditary nature of the syndromes closely related to vitamin E deficiency in animals and in man. A mutation of such a regulatory gene in the hereditary disorders could make its repressor protein insensitive to a-tocopherol and account for the similarity in clinical and biochemical findings in these diseases as well as the ineffectiveness of vitamin E as a therapeutic agent.

¹⁴A. L. Tappel, "Where Old Age Begins," Nutrition Today, 2:4:6-7, December, 1967.

¹⁵Robert Olson, "Are We Looking at the Right Enzyme Systems?" The American Journal of Clinical Nutrition, 20:6:609, June, 1967.

¹⁶H. V. Gelloin, "Carcinogens, Enzyme Induction and Gene Action," Advances in Cancer Research, Vol. 10 (New York and London: Academic Press, 1967), p. 609.

His conclusion states that

...vitamin E acts as a "repressor" for the catabolic enzymes...The increased metabolic turnover uniformly noted in vitamin E-deficient animals is, in my opinion, due to increased content of catabolic enzymes. Creatinephosphokinase is not, inherently catabolic, but it appears to be redundant in vitamin E deficiency and in hereditary muscular dystrophies in which repressor proteins may be absent or altered. The fact that elevated CPK values in vitamin E-deficient rabbits are promptly corrected by an inhibitor of protein synthesis as well as by tocopherol, supports the view that its turnover is increased in such animals and is decreased by the vitamin.¹⁷

And so the fifth function of vitamin E is the repression of the catabolic enzymes of the lysosomes.

Protects Vitamin A. In connection with the antioxidant function and the influence on the reproduction system, Keele and Horwitt state that vitamin E might protect vitamin A. Proufit states this is accomplished by minimizing the oxidation of carotene and vitamin A in the intestine. If vitamin E is depleted then vitamin A would be oxidized, in turn, creating the symptoms of vitamin A deficiency.

Olson lists the vitamin A functions as: 1) a photoreceptor; 2) the growth and maintenance of epithelial tissues; and 3) influencing mucous secretion. Keele states that vitamin A deficiency delays sexual maturity, and contributes to infrequency of ovulation; "there is extensive keratinization of the vagina and continuous shedding of the horny cells which obscure the normal changes which occur during the oestrous cycle."¹⁸

¹⁷Olson, loc. cit.

¹⁸Keele, op. cit., p. 527.

Summary of Vitamin E Functions. Olson summarizes the functions of vitamin E as (1) antisterility and progestational, (2) antianemic, (3) antioxidant, (this includes its function as a protector of vitamin A) (4) the developing and maintaining of muscle integrity, and (5) the repressing of the catabolic enzymes of the lysosomes.

D. Manifestations of Vitamin E Deficiency in Animals

A bewildering array of studies of vitamin E deficiency in animals has been done over the past forty years. Horwitt states in his review that "it is apparent that the structural integrity of muscle, the reproductive organs, the nervous system, the vascular system, and the glandular tissue all can be involved." Early manifestations of the deficiency seem to be species specific. Different tissues or systems are affected first in the different animals used to test vitamin E deficiency. Because of the ease with which it can be produced in the rat and the rabbit, these animals have been used frequently in the laboratory.

Species Specificity. Vitamin E deficiency shows species specificity. For example, in calves, lambs and rabbits muscular deterioration is among the first symptoms. It progresses to muscular dystrophy. However, red cell instability is seen first in the rat, followed by the failure of the reproductive system.¹⁹ The irregularities of the central nervous system of the chicken is a consistent manifestation; while in the turkey, the gizzard is affected.²⁰ In the

¹⁹Harris, op. cit., pp. 602, 603.

²⁰Ralph W. Gerard, Food For Life (Chicago: University of Chicago Press, 1952), pp. 242, 243.

rabbit and cow the heart shows signs of vitamin E deficiency²¹ and monkeys have a shortened red cell survival with "bone marrow morphology."²² In humans hemolytic anemia occurs first and is followed by many other manifestations of the deficiency.

Many Tissues and Systems are Affected. As seen in the manifestations above, both striated and smooth muscles can be involved when an animal is vitamin E deficient. Both the blood cells and nerve cells are involved. The pathology in various organs and systems have been noted above. Recorded experiments indicate the pathological involvement of other organs than those mentioned here. Liver and kidney involvement were noted by Wertz. "Necrotic degeneration of the liver can be produced in rats by feeding them a diet deficient in cysteine and vitamin E."²³ "...prenatal vitamin E deficiency affected the normal chemical development of the embryonic liver."²⁴ Endocrine involvement is shown in experiments that demonstrated "a reduced thyroid activity index,"²⁵ and a depressed thyroid stimulating hormone (TSH) secretion of the pituitary

²¹J. M. Wertz, C. R. Seward, E. L. Hove, and J. S. Adkins, "Inter-relationship of Dietary Glycine, Methionine and Vitamin E in the Rat," The Journal of Nutrition, 94:2:129-138, February, 1968.

²²Oski and Barness, op. cit., p. 218.

²³W. A. Sebrell, and Robert Harris, The Vitamins, Vol. II (New York: Academic Press, 1954), p. 75.

²⁴Dorothy Weiking, and Kusum Verma, "A Histochemical Study of Embryonic Rat Liver in Avitaminosis E^{1, 2, 3}," The Journal of Nutrition, 91:1, Part I:159, February, 1967.

²⁵W. Boguth, and M. Sernetz, "Thyroid Function of Rats on a-tocopherol- and Ubiquinone--Deficient Diet," (University Giessen, Germany, Int. Z. Vitaminforsch., 1967), pp. 412-415, as cited in Chemical Abstract #66893d.

gland in rats.²⁶

In a report at the 19th annual proceedings of the Montana Nutrition Conference for feed manufacturers, dealers, salesmen and livestock men at Montana State University, Bozeman, Montana, held February 5-7, 1968, C. R. Adams summarized the types of conditions related to vitamin E deficiency in animals that have been reported in literature, i. e. :

- Testicular degeneration
- Embryo degeneration
- Exudative diathesis
- Anemia
- Red blood cell fragility
- Muscular degeneration
- Encephalomalacia
- Necrotic liver degeneration
- Kidney tubule degeneration
- Stomach ulcers
- Steatitis (yellow fat disease)
- Oxidative rancidity in meat and milk
- Bone deformity
- Depigmentation of teeth
- Nerve degeneration
- Impaired utilization of vitamin A and carotene
- Impaired utilization of protein

Adams concludes that:

...these conditions may occur from other causes, but one should not overlook the possibility of insufficient vitamin E intake as a possible cause.... There is a growing feeling that marginal deficiencies of vitamin E, which seldom manifest themselves in easily detectable symptoms, are more common among domestic animals than previously believed.²⁷

²⁶W. Boguth, R. Repges, and M. Sernetz, "Thyroid Function in Rats Fed a Diet Deficient in Tocopherol and Ubiquinone," (University Giessen, Germany, Int. Z. Vitaminforsch., 1967), pp. 287-292, as cited in Chemical Abstract #66893d.

²⁷C. R. Adams, "A Report" at the 19th Annual Proceedings of the Montana Nutrition Conference for feed manufacturers, dealers, salesmen and livestock men, Montana State University, Bozeman, Montana, February 5-7, 1968, p. 42.

In regard to the findings documented in this section, this investigator wonders, "Why is vitamin E deficiency so selective? Why are the manifestations so varied?" The study of many experiments and related literature begins to reveal a pattern.

The Balance of Polyunsaturated Fatty Acids and Vitamin E. The ratio of polyunsaturated fatty acids (PUFA) to vitamin E seems to be a factor in the selectivity and varied manifestations of vitamin E deficiency. Horwitt thinks that if investigators would evaluate the polyunsaturated fatty acid content of the tissues simultaneously when investigating the concentration of vitamin E that "many of the conflicting reports now in literature might be brought to a harmonious synthesis." He states that the "stores of different fatty acids in the hen will affect the composition of the lipids of the egg which, in turn, may make the brain of the chick more or less susceptible to encephalomalacia--a morbid softening of the brain." He also states that the high polyunsaturated fatty acid content of the rat testical accounts for the "specificity of the action of vitamin E with regard to testicular degeneration. . . (and) that even on a low fat diet in which the need for a-tocopherol by other tissues is very low, the testes of the rats have high levels of polyunsaturated fatty acids."²⁸

Enzyme Control. Cells as primary units of tissue are made up of the components of the cell and each process is influenced by an enzyme (Appendix B). Enzymes require the right proportion of constituents and influence metabolic pathways. "There are many enzyme systems in which lipoprotein is a component part. Since each of these lipoproteins can be altered to contain a higher

²⁸Horwitt, op. cit., pp. 7-9.

proportion of autooxidizable PUFA (polyunsaturated fatty acids) it is no wonder that so many different and unrelated enzyme systems are affected when the ratio of PUFA gets out of balance. "²⁹

Since the change of position of one amino acid in the enzyme proteins can be transmitted to future generations as an inborn error, the possibility of familial traits in enzymes that trigger the processes of metabolic pathways need to be considered as a factor influencing the many possibilities related to vitamin E deficiency.

Research is concerned with which enzymes and which metabolic pathways may be involved. One study of the swollen axons in vitamin E deficient rats indicated the possibility of a deficiency of "succinic dehydrogenase in the mitochondria of the swollen axons. "³⁰ Another researcher, using vitamin E deficient guinea pigs, observed "an increase in fast moving peak of myoalbumin, (an) increase in the fraction with phosphorylase activity, (and an) increase in proteins with aldolase and triose phosphate dehydrogenase activity. "³¹

Summary of Vitamin E Deficiency in Animals. In summarizing vitamin E deficiency in animals, it is of value to note that the selectivity and variation in the manifestation of vitamin E deficiency seems to be related to the

²⁹Ibid., p. 9.

³⁰Stirling Carpenter, "A Histochemical Study of Oxidative Enzymes in the Nervous System of Vitamin E Deficient Rats, " Neurology, 15(4), 328-332, (Washington, D.C.: Armed Forces Inst. of Pathol., 1965), as cited in Chemical Abstract, Vol. 63, #979g.

³¹I. I. Ulsevich, "Influence of Water Soluable Analog. of Vitamin K on Changes in the Sarcoplasmic Proteins of Muscles Developing in Vitamin E Deficiency, " Byul. Eksperim. Biol. i. Med., 59(7), 54-56, 1965, (in Russian), as cited in Chemical Abstract, Vol. 63 (1965), #12178e.

(1) polyunsaturated fatty acid content of various tissues, and (2) to enzyme control--(a) through a balance of vitamin E and polyunsaturated fatty acids in the cell or tissues and (b) through hereditary factors related to enzymes.

The possibility that the species specificity of vitamin E deficiency would eliminate the human from any manifestation has provided false hope. There is now evidence of vitamin E deficiency in humans.

E. Vitamin E Deficiency in Humans

Vitamin E deficiency in humans seems to be as varied as in animals. Malabsorption syndromes with steatorrhea (fatty stools), hereditary factors, and pigmentation seem to have special significance. Signs of vitamin E deficiency occur in all age groups and in both sexes.

Variation. "...in some deficient patients, creatinurea, ceroid deposition in smooth muscle and focal necrosis of striated muscle have been observed."³² Oski also observed hemolysis in premature infants who exhibited evidence of vitamin E deficiency. In some adults who had malabsorption syndromes there was evidence of vitamin E deficiency along with shortened red cell survival; and, in some, Heinz bodies could be demonstrated in the absence of a specific enzyme.³³

In a study done by Potoskii twenty patients of thirty-three studied who had eczema in the period of dentition, menstruation, gestation, lactation, or

³²Oski, op. cit., pp. 211-220.

³³Henry J. Binder, and Howard M. Spiro, "Tocopherol Deficiency in Man," The American Journal of Clinical Nutrition, 20:6:594-599, January, 1967.

climacteric (stressor periods) had increased sedimentation of the erythrocytes, and the vitamin E content in their blood serum was considerably below normal. Biochemical tests indicated participation of endocrine glands in the development of eczema in a number of patients.³⁴ Eczema is sometimes associated with either the deficiency of, or malabsorption of, polyunsaturated fats. Horwitt's review reveals that there is a relationship between vitamin E serum levels and polyunsaturated fats.

In a study done in Uganda, thirty per cent of the patients picked randomly in a clinic were found to be deficient in vitamin E. There was a high incidence of malnutrition, cerebrovascular accidents and liver disease among the deficient patients.³⁵

Cerebellar pathology in a fourteen month old infant was reported by Horwitt. "It had the same histopathological lesions as found in the cerebellum of chicks with nutritional encephalomalacia."³⁶ Binder found neuropathy associated with steatorrhea--excessive loss of fat through the feces.

The multiple manifestations of vitamin E deficiency in humans may be related to other phenomena and conditions.

Malabsorption Syndromes. Binder and Spiro state that "tocopherol depletion not infrequently occurs in humans with steatorrhea," and Oski cites a

³⁴I. I. Pototskii, "Effect of the Endocrine Glands on the Course of Eczema," Vrachebnoe Delo, (7), pp. 90-94, (in Russian), (Kiev, Russia: Mediat Inst., 1965), as cited in Chemical Abstract Vol. 63, #12114b.

³⁵J. A. Tulloch, and N. K. Snood, "Vitamin E Deficiency in Uganda," The American Journal of Clinical Nutrition, 20:8:884-887, August, 1967.

³⁶Horwitt, op. cit., p. 11.

study by Gordon and Nitowski to support the idea that vitamin E deficiency is the consequence of prolonged malabsorption of fat. Vitamin E, being a fat soluble vitamin, could be lost through the feces. Thirty-five subjects with various diagnoses but whose common denominator was the presence of steatorrhea were studied. When steatorrhea is present, there is a loss of blood serum tocopherol stores that precedes the loss of erythrocyte tocopherol stores. The duration of the depletion or the presence of steatorrhea "does appear to be important for the occurrence of more severe tissue depletions." Binder also stated that tocopherol is normally found in the mitochondria and suggested the possibility that "its absence could lead to abnormal cellular respiration, defects in lipogenesis, and accumulation of various lipid products."³⁷

Green and Goldberger have noted that malabsorption syndromes are related to heredity factors or a defective protein or enzyme as coded by DNA.³⁸

Hereditary Factors as a Predisposition to Disease. Protein structures are determined by DNA. If any part of the code is altered by intrinsic or extrinsic factors, a mutation or altered code can, and sometimes does, occur. If this alteration is relative to a major protein or tissue, deformity or death may occur to the generated organism. However, a minor change can occur relative to an amino acid sequence in a peptide chain with the organism maturing to adulthood apparently normal. Precipitating incidents may be necessary to trigger a pathological condition. This is hereditary predisposition to disease and the characteristic is transmitted via DNA from generation to generation. For

³⁷Binder and Spiro, loc. cit.

³⁸Green and Goldberger, op. cit., pp. 344-345.

example, sickle cell anemia is a condition in which the red blood cells become sickle shaped; this occurs when the hemoglobin within them becomes deoxygenated and remains deoxygenated for a long enough time and individuals with one type of hemoglobin (HbS) develop these sickled cells that precipitate out of solution. This is due to the change in position of one base coded in at the DNA level and determines the degree of solubility of the hemoglobin.³⁹ In this way, some familial groups have a type of hemoglobin that is susceptible to damage resulting in deoxygenation and precipitates sickled red blood cells.

Vitamin E deficiency seems to be related to some inborn error or errors. In Binder's study, in adult patients who manifested shortened red cell survival, malabsorption syndromes, and evidence of vitamin E deficiency, Heinz bodies could be demonstrated in the absence of glucose-6-phosphate dehydrogenase--an enzyme. Green and Goldberger list several genetic defects that influence red cell metabolism. The deficiency of glucose-6-dehydrogenase is a genetic defect in the protein that involves red cell metabolism causing a disease known as favism. Another genetically defective protein or enzyme is one that involves pyruvate kinase which also involves red cell metabolism and contributes to hemolytic anemia.⁴⁰ Oski cites the authority of Kayden to state that patients who suffered with acanthocytosis--a hereditary defect in beta lipoprotein synthesis--were shown to be markedly vitamin E deficient.

Creatine phosphokinase (CPK) is excreted in the urine of some vitamin E deficient persons such as hereditary muscular dystrophies. "CPK is not

³⁹Ibid., pp. 334-340.

⁴⁰Ibid., p. 345.

inherently catabolic, but it appears to be redundant in vitamin E deficiency and in hereditary muscular dystrophies in which repressor proteins (or enzymes) may be absent or altered. The fact that elevated CPK values in vitamin E deficient rabbits are promptly corrected by an inhibitor of protein synthesis, as well as by tocopherol, supports the view that its turnover is increased in such animals and is decreased by the vitamin. ⁴¹

There are several varieties of primary familial lipoidosis--a faulty lipid metabolism--that are influenced by enzymes or proteins and become defective genetically. ⁴² However, little is known about these at this time. Could the steatorrhea associated with vitamin E deficiency be the result of one of these familial lipoidosis?

Researchers continue to devise new methods of research and to integrate known facts into new concepts. Recently, one researcher produced, by inbreeding, a strain of mice that was more susceptible to vitamin E deficiency. The pathology of the hereditary strain had an etiology independent of the strain that developed pathology due to nutritional deprivation of vitamin E. ⁴³ This may provide one explanation for the varied results of authentic research that has puzzled researchers.

An observation of some researchers interested in vitamin E deficiency

⁴¹Olsen, op. cit., p. 60.

⁴²Green and Goldberger, op. cit., p. 345.

⁴³Rolf Loosli, "Hereditary and Nutritional Muscular Dystrophy in an Inbred Strain of Mice," (Jackson Lab., Bar Harbor, Maine), Journal of Heredity, 56(2), 1965, pp. 75-81, as cited in Chemistry Abstracts, Vol. 63, #6071f.

is that often there is pigmentation in various tissues, (such as fatty deposits, heart muscle, nerve cells, uterine muscle, etc.), of animals and humans who show clinical evidence of this deficiency. Could this pigmentation be related to chloasma gravidarum in any way?

CHAPTER IV

PIGMENTATION

A. Facts Concerning Pigmentation

Melanin. Dorland's Medical Dictionary states that melanin is "the dark amorphous pigment of the skin, hair, and melanotic tumors, of the choroid coat of the eye and the substantia nigra of the brain. It is a dihydroxylic acid.... It is a product of cell activity, contains sulfur and iron, and is probably a derivative of hematin." Gray's Encyclopedia of the Biological Sciences states that "melanin may be produced by the interaction of an oxidase (tyrosinase) with tyrosine (an amino acid from which adrenalin is also derived--see page 58, Figure 1)," and that it is one of three "surface pigments of animals--carotinoid, melanin and guanine--that may be derived from food or from endogenous metabolism."⁴⁴

Melanin Promotes Non-enzymic Oxidation of Intermediates. A recent experiment reveals that "melanin promoted the non-enzymic oxidation of NADH (the reduced form of NAD) to NAD (nicotinamide adenine dinucleotide)--(intermediate molecules of a metabolic process) in vitro. The oxygen uptake was higher when influenced by light. One atom of oxygen was consumed per mole of coenzyme oxidized. The reduced melanin was subsequently autooxidized producing melanin and hydrogen peroxide. (underlining mine) The formation of hydrogen peroxide suggested a 2-electron step in melanin. The stable free

⁴⁴Cleveland P. Hickman, Gray's Encyclopedia of the Biological Sciences. (New York: Peter Gray Reinhold Publishing Corporation, 1961), pp. 774-775.

radical property of melanin may serve a metabolic function in the melanocyte."⁴⁵

Other Pigments. Gray's Encyclopedia lists other pigments that may account for the pigmentation of tissues as being the chromolipoids (yellows or darkish-brown of ovaries and adrenal glands), flavins (yellow green fluorescence of many glandular tissues exposed to ultraviolet light), and the pyrroles (represented by the chlorophyll of green plants and by hemoglobin which contains the heme pigment--both important in respiration but usually playing minor roles in the surface pigmentation).

The Melanocyte. The melanocyte is--according to Dorland's Medical Dictionary--"the epidermal cell which synthesizes melanin; constituting five percent of the epidermal cells and, with the keratinocyte, forming the binary cell system of the epidermis." Melanoderma occurs when there is "an increased amount of melanin in the skin, due either to an increase in the production of melanin by the melanocytes normally present or to an increase in the number of melanocytes, with production of hyperpigmented patches."

Lerner states that

Melanocytes are nerve cells. Embryologically they are derived from the neural crest. Biochemically they behave as nerve cells. Since... MSH (melanin stimulating hormone) in minute amounts evokes dramatic responses in melanocytes, its action on different neural structures should be determined. It is even possible that the control exerted by MSH on melanocytes is less important than its effect on other neural organs.⁴⁶

⁴⁵M. H. Van Woert, "Oxidation of Reduced Nicotinamide Adenine Dinucleotide by Melanin," Chemical Abstracts #65781d, 1967, pp. 2605-2612.

⁴⁶Aaron B. Lerner, "Possible Physiological Function of Intermediate Lobe Hormones in Mammals," The Pituitary Gland, Vol. III, Ed. G. W. Harris and B. T. Donovan, (Los Angeles: University of Calif. Press, 1966), pp. 33-41.

The adrenal medulla is a neuro-hormonal mechanism. Does increased MSH production influence its function?

The Pituitary Gland's Function in the Production of Melanin. The pituitary gland is about the size of a pea and is located in the center of the head next to the hypothalamus. It is composed of three lobes. The intermediate cells (or lobe) are not clearly differentiated from the other lobes but are attached to the posterior lobe. These cells produce the melanocyte-stimulating hormones (MSH) a- and B-MSH, which are peptides related in structure to ACTH from the anterior lobe.⁴⁷

...It is possible to use human subjects to assay for skin darkening peptides such as MSH and ACTH. Medium colored Negroes given repeated intra-muscular injections of the peptides respond in one to twelve days with generalized skin darkening that is most striking on the face... (measurement of MSH in the blood and urine has been done since 1933). Four findings among the investigators are as follows: 1) MSH activity is increased in the blood and urine of patients and animals with adrenocortical insufficiency; 2) MSH is decreased in man after hypophysectomy (removal of the pituitary gland); 3) administration of steroids decreased in man after hypophysectomy; 4) MSH in the urine increases during pregnancy. (underlining mine) MSH in the blood varies from 0 to 4 units per ml. and in the urine from 20 to 100 units per 24 hour specimen. It is not yet possible to use MSH determinations as an indication of pituitary function. If it becomes possible to separate and determine quantitatively a- and B-MSH and ACTH in the blood or urine of patients with different disorders, a valuable test of pituitary function will have been achieved. Then we shall know the proportionate role played by each peptide in the skin darkening of patients with adrenocortical insufficiency or pituitary tumours.⁴⁸

Significant for this paper are findings 1 and 4. According to Russo

⁴⁷J. F. Christ, "Nerve Supply, Blood Supply and Cytology of Neuro-hypophysis," The Pituitary Gland, Vol. III, Ibid., p. 74-f. (Recent experiments indicate that the posterior lobe is related to the production of ACTH.)

⁴⁸Lerner, op. cit., pp. 28-33.

The most accurate and dependable methods of assay available today do, however, indicate that pregnant women and victims of Addison's disease excrete about ten times the normal amount of MSH per day.⁴⁹

The increased pigmentation and increased MSH in the urine that is seen in pregnancy and Addison's disease need additional investigation.

Intermediate Lobe Hormone Functions. Lerner states that

In man, daily injections of 8 mg. of a- or B-MSH or 24 mg. of ACTH bring about pronounced darkening in 7 to 12 days.... (and it is) suggested that the dark skin of people in Africa provides superb camouflaging in the forests. Perhaps in this sense MSH is a vestigial hormone. However, it may be that small amounts of MSH allow human melanocytes to exist in a some what darkened state which offers protection against sunlight. When large amounts of MSH are released by the pituitary gland of patients suffering from adrenocortical insufficiency or some type of pituitary tumor, marked darkening of the skin occurs.... There are many indications that the pituitary gland contains a fat-mobilizing hormone. As pure pituitary hormones become available, they are tested for effects on lipid metabolism. a- and B-MSH have been found to increase the circulating free fatty acids in rabbits and to cause the release of pituitary peptides, including ACTH, have the same effect but to different degrees. For example, with rabbit fat pad tissue a-MSH is more active than ACTH.... Peptides called Fraction H and Fraction L, ...and Peptide I and Peptide II... may be specific for mobilizing fat. These substances have not been found to have any other activity either in vivo or in vitro. Presumably they are produced in the anterior lobe of the pituitary gland. Their structures are not yet known.... The effects of a- and B-MSH on free fatty acids in the blood of man have not been determined. Yet it was discouraging to find that huge quantities of ACTH... injected intramuscularly into a human subject produced little if any change in the blood fatty acid level.⁵⁰ (underlining mine)

Another function is suggested by Lerner. "...intravenous administration of B-MSH to cats increased, for long period of time, the amplitude of mono-synaptic potentials in the spinal cord. Neither a-MSH nor ACTH produced this change."⁵¹

⁴⁹ John Paul Russo, "... Take As Directed," edited by F. E. Shideman (Cleveland, Ohio: CRC Press, 1967), p. 325.

⁵⁰ Lerner, op. cit., pp. 59-62.

⁵¹ Lerner, loc. cit.

The functions of MSH include 1) the camouflaging or protection against sunlight; 2) possible fat-mobilizing function; and 3) the possible amplification of monosynaptic potentials in the spinal cord. In other words, MSH does influence skin pigmentation and may effect fat metabolism and the nerve synapses.

MSH Function is Influenced. Human MSH (melanin stimulating hormone) is made up of a twenty-two amino acid sequence. The a-MSH stimulating activity is less than B-MSH. Structure variation is possible by adding or removing certain amino acids which activate or inactivate MSH. Hydrogen peroxide and the thiols inactivate and reactivate MSH.⁵²

Research continues to probe the secrets of the pituitary gland and the hypothalamus between which there is interaction. There is, now, some question concerning the function of each. Electrical stimulation of different areas of the hypothalamus produces the secretion of various hormones such as the gonadotrophic hormones.⁵³

Sex Hormones Sensitize the Skin to MSH Action. Cooley states that "injection of MSH (melanin stimulating hormone) causes the skin to darken in 24 hours--but not if the subjects are eunuchoid men with little androgen production. Sex hormones appear to sensitize the skin to MSH action. This interaction explains the skin darkening of some women during pregnancy or at phases

⁵²Ieuan Harris, "The Chemistry of the Intermediate Lobe Hormones," The Pituitary Gland, Ibid., pp. 33-41.

⁵³J. F. Christ, "Nerve Supply, Blood Supply and Cytology of the Neurohypophysis," The Pituitary Gland, Ibid., pp. 63-131.

of the menstrual cycle."⁵⁴ Another note of interest is the fact, that some women who take birth control pills often manifest this skin darkening. Also, the phenomenon may recur at menopause for some women. But for a few this skin discoloration fades temporarily, only to reappear in various degrees throughout life.

Griffen, the author of Black Like Me, achieved a darkened skin by the use of medication he obtained from a dermatologist. He toured the South as a negro and then wrote his book. According to news media, he died a few years later due to the chemicals he had used to darken his skin.⁵⁵

B. Tissue Pigmentation and Lipid Oxidation

Isolated mention of the observation of tissue pigmentation relative to vitamin E research has been recorded incidentally throughout the more than forty years in which researchers have tried to find a disease for this possible deficiency. Horwitt devotes a section of his review to "Tissue Pigmentation Related to Lipid Oxidation."⁵⁶

Pathological Pigment. Horwitt gives credit to Martin and Moore for first calling attention to the occurrence of a "brownish, acid fast pigment in the uterine muscle, skeletal muscle, sex glands, and other tissues of rats maintained on vitamin E deficient diets." He states that

⁵⁴Donald G. Cooley, "Hormones: Your Body's Chemicals," Today's Health, 40:30, December, 1962.

⁵⁵John Howard Griffen, Black Like Me (Boston: Houghton Mifflin Company, 1961).

⁵⁶Horwitt, op. cit., pp. 6, 7.

this was soon confirmed by other investigators, who also found the brownish discoloration of affected tissues. Similar discolorations were observed in the monkey, hamster and rat. The fact that the compound had a brownish-yellow fluorescence was recognized by Moore and Wang in 1943. Since those early studies, reports have accumulated. They tend to agree that this pigment is indistinguishable from the "ceroid" pigment isolated by Lillie, et al., from livers of rats with nutritional cirrhosis. Apparently it is a product of auto-oxidation and condensation of polymerization of polyunsaturated lipids with other tissue products.

The Need for Vitamin E Identified. Horwitt continues:

One of the clearest presentations of this relationship between the auto-oxidation of polyunsaturated fatty acids (PUFA) and vitamin E deficiency was presented by Filer, et al., in 1946. They discussed the requirement for tocopherol in the light of the theory of Longenecker and Tiemenschneider that the synthesis of glyceride fat in the body results in a distribution of fatty acid representative of the diet. Dietary fat has a great effect on the amount of individual unsaturated fatty acids that tie up with glycerol to form the depot fats. They associated this dietary change with the varying needs of the body for protection by antioxidants.

The Pigment of Vitamin E Deficiency. The examination of the vitamin E deficient pigment in the electron microscope revealed that "this pigment does not originate from preformed cytoplasmic organelles nor has it any constant ultra-microscopic structure. It is transformed morphologically (pigments can be formed from endogenous metabolism--see page 32) in the course of aging, in parallel with the oxidation and polymerization of the lipids deposited."⁵⁷
(underlining mine)

Tappel states that "cell clinkers are formed by the remains of lysosomes which attract and hold enzyme stain. They emit a rather muddy yellow fluorescence." These clinkers are the pigments that remain after the attack by free radicals and oxidation of cell components in the absence of antioxidants.

⁵⁷P. Gedigk, "Formal Genesis of the Vitamin E Deficiency Pigment in the Electron Microscope," Verhandl Deut. Ges. Pathol. (University of Murburg, Germany, 1963), 47:258-260, as cited in Chemical Abstracts, Vol. 63, #3381g, 1965.

Lipofuscin and Intrinsic and Extrinsic Factors. In 1961 Horwitt pointed to the possibility of both intrinsic and extrinsic factors relative to aging and the deposition of the age pigment.

Radiant Energy, An Extrinsic Factor, Speeds Up the Normal Aging Process. In 1967 Dr. Tappel pieced together a theory of aging (Appendix B).

The aging of our bodies appears to be influenced by an intracellular tug of war going on between two factors acting on a third: intensity and duration of radiation-like effects; polyunsaturated lipids upon which they act; and the vitamin E available to protect the lipids from excessive destruction... evidence has also been discovered that these same beneficial fats (polyunsaturated fats) may well be a source of radicals within the cell that cause it to age. For example, when radiant energy, which can penetrate throughout the body entering every cell, strikes a polyunsaturated lipid that is present as a nutrient, one of two things happens. If enough vitamin E is present, the radiation will have little effect. If, however, there is an intracellular deficiency of vitamin E, the energetic rays will strike a lipid molecule and knock loose a hydrogen atom. This would typically initiate the peroxidation of polyunsaturated lipid. Peroxidation involves the direct reaction of oxygen and lipid to form free radical intermediates. The free radical flies about within the cell under terrific force and without any pattern to its movements until it strikes another molecule and causes all sorts of damage. Lipid peroxidation is, therefore, widely regarded as the main spring in the aging process.

Free radical damage isn't just an isolated incident; it is occurring all the time in the body...and when enough cells are affected, the host looks old and soon succumbs to disease. These events all take place at the most fundamental level of cellular physiology because they begin with molecular reactions involving vital cell constituents....

Because the mitochondria and the endoplasmic reticulum contain a great deal of polyunsaturated lipid, they are very vulnerable to the peroxidizing effect...they swell abnormally, disintegrate and dissolve. Because the mitochondria are the powerhouses of the cell, effective lipid peroxidation knocks out the energy-generating apparatus of electron transport and phosphorylation. It is of significance that this end result can be seen if 1) the animals are deficient in vitamin E, or 2) if they are aged, or 3) if they have had a very heavy dose of radiation. (underlining mine)

Equally vulnerable are the...lysosomes...(that dissolve) cellular waste materials. This debris reduction process is accomplished by acidic hydrolytic enzymes...(that are released) into the cell, destroying other cellular components as well as the lysosome itself....it seems certain that

the very lethality of the lysosomal enzymes and their ability, in effect, to compound the damage already done to a cell by peroxidation set the pattern by which a few free radicals released by radiation-like processes are able to produce the devastating sequelae that we can observe in gross pathology. It seems the prelude to the tissue catabolism that we associate with old age.

The function of the lysosomes in the cell involves them in cellular geriatrics in a second manner....even if their membrane remains intact and destruction is taking place all around them,....an excessive amount of debris accumulates,...(and) the lysosome takes in waste products. Once inside the membrane, enzymes hydrolyze most of the polymeric waste such as proteins, polysaccharides, and nucleic acids. However, when this system is overloaded, waste dissolution slows down and stops and...the lysosome founders and the cell that houses it drowns in its own waste.⁵⁸

The Cell Clinker: A Clue to Cellular Age. The decisive clue to cell age is the

ash or "clinker" that remains when the cell has been burned out by peroxidation. These clinkers are colored pigments that remain after the attack by free radicals....Analysis of these clinkers reveal that they contain some enzymes from both lysosomes and mitochondria indicating that damaged mitochondrial membranes could be a major contributor to the composition of the age pigments.⁵⁹ (see Appendix B)

Horwitt's Discussion of Lipofuscin--The Age Pigment.

Returning to the discussion of pigments that may be related to oxidation of lipids, "lipofuscin" was noted in heart muscle tissue by Stubel in 1911, and by Bommer in 1929. Hamperl used fluorescence microscopy to study this pigment. He observed that it was absent in subjects below age ten. Strehler, et al., in a recent paper reviewed past work on the "age pigment" and reported on a study of the quantitative relationship of lipofuscin to age. Somewhat amazing is the observation that as much as 10% of the intracellular volume of the heart muscle cells may be occupied by this pigment.

Strehler, et al., confirmed the fact that this pigment is not apparent in heart muscle during the first 10 years of life, but is found in the hearts of

⁵⁸A. L. Tappel, "Where Old Age Begins," Nutrition Today, 2:4:2-5, December, 1967.

⁵⁹Ibid.

all older individuals. The concentrations found were independent of sex, race or pathology.⁶⁰

Can Stress Produce Lipofuscin? Horwitt discusses the possibility of stress and the production of lipofuscin (the age pigment).

Hodge had noted in 1894 that the nerve cells of the fetus contain almost no pigment, and that the cytoplasm of nerve cells from senile humans was largely filled with pigment. . . . Wolf and Pappenheimer suggested a relationship between this nerve cell pigment and the substance called "ceroid" which Lillie, Daft and Sebrell had found in the livers of vitamin E deficient rats. The deposition of such pigments in the nervous system of vitamin E deficient rats was first described by Einarson and Ringsted. . . , data were presented to support the view that lipofuscin pigments in nerve cells can be produced experimentally in rats by factors other than aging. Thus, vitamin E deficiency produced a greater degree of deposition of lipofuscin pigment in the cytoplasm of the nerve cells of young rats, . . . Also of interest was Sulkin's observation that experimental conditions which have been associated with stress factors in vitamin E research, i.e., ACTH administration, anoxia, and the feeding of excessive amounts of acetanilide to raise methemoglobin concentrations; all increased the deposition of lipofuscin pigment in nerve cells.

Studies on Aging by Selye. Bailey cites a published paper in which Selye reported some experiments relative to stress (see Appendix B), aging and treatment with vitamin E. He produced evidence of aging in rats and compared it to a control group of rats. Then he produced aging in two groups of rats and treated one group with large doses (1,000 mg.) of vitamin E. The treated rats were able to withstand the aging process while the untreated rats died from the noxious treatment.⁶¹

⁶⁰Horwitt, loc. cit.

⁶¹M. Cantin, Jean-Marie Diendonne, and H. Selye, "Effects of Vitamin E on Cardiomuscular Calciphilactic Lesions," Exp. Med. and Surg. (Canada: 1962), 318:20, as cited by Herbert Bailey, Vitamin E: Your Key to a Healthy Heart (New York: ARC Books, Inc., 1964), pp. 94-96.

C. Pigmentation in Various Tissues

Brown Fat. Vitamin E-deficient rats given unsaturated fatty acids "show a diffuse brown pigmentation of stored fat, together with a lack of the normal pigment in the incisors which is due to iron. The pigmentation of lipids is taken to indicate a metabolic interrelationship of vitamin E and the essential fatty acid deficiency..."⁶²

Dark Pigments in Urine. Dark pigments have been noted in the urine of persons with hemolytic disease associated with acute episodes of hemolysis.⁶³

The Brown Bowel and Deposition in Other Smooth Muscles. Binder and Spiro, as the result of a study done on tocopherol deficiency in man, state that further pathologic evidence of tocopherol deficiency does exist. The PAS-positive (ceroid-like) material has been found in smooth muscle of tocopherol-deficient individuals. This has been identified in patients with chronic pancreatitis, sprue, and cystic fibrosis. Interestingly enough, it has never been found in a dying child with cystic fibrosis under 2 years of age and has always been found in children over 5 years of age. We have found evidence of ceroid deposition in four subjects, two of whom had such marked deposition of PAS-positive material as to lead to a brownish discoloration of the serosal surface of their small intestine. (underlining mine) This gave the appearance of a "brown bowel." In two subjects studied at postmortem, PAS-positive material was demonstrated in almost all smooth muscles examined, including urinary bladder and heart muscle. The PAS-positive material was found in the muscularis mucosae of rectal biopsies of two patients. This suggests an easy method for documenting pathologic tocopherol deficiency, i.e., the rectal biopsy.⁶⁴

⁶²Ralph T. Holman, The Vitamins, edited by W. H. Sebrell, Jr., and Robert S. Harris (New York: Academic Press, Inc., 1954), p. 316.

⁶³M. D. Prankard, "The Over-All Significance of Metabolism and Enzyme Activity in the Red Cell," The American Journal of Clinical Pathology, 47:3:285, 1967.

⁶⁴Binder and Spiro, op. cit., pp. 594-601.

Pigments in the Brain of Neurotoxic Animals.

Mitochondria from the whole brain or cerebellum of newborn guinea pigs with experimental bilirubin encephalopathy failed to exhibit uncoupling of oxidative phosphorylation. The pigment concentrations required to initiate uncoupling in vitro are much higher than those found in the brain of neurotoxic animals.... The present observation suggests that uncoupling of oxidative phosphorylation may not be the decisive biochemical event underlying pigment toxicity in experimental bilirubin encephalopathy.⁶⁵
(underlining mine)

D. The Time Factor

Binder and Spiro also state that "it would appear from our material that the occurrence of PAS-positive material (ceroid-like material or lipofuscin) is determined by the duration of tocopherol deficiency. Serial biopsies were available in two of our subjects. The PAS-positive material was identified only after an interval of at least three years."⁶⁶

E. Pathological Conditions with Pigmentation as a Manifestation

MSH (melanin stimulating hormone) influences skin pigmentation and may effect fat metabolism as well as the nerve synapses (see page 35). There are some conditions and diseases known to medical science in which pigmentation is a characteristic symptom. A few of these were listed and then the other symptoms examined to see if these symptoms are repeated in other conditions in which pigmentation is a symptom. Do conditions with pigmentation also have symptoms related to faulty fat metabolism? Are their symptoms related to

⁶⁵Ivan Diamond, and Rudi Schmid, "Oxidative Phosphorylation in Experimental Bilirubin Encephalopathy," (abstract) in Science, 155:1288-1289, March, 1967.

⁶⁶Binder and Spiro, op. cit., pp. 594-601.

amplification of nerve synapses? Are they repetitious of the clinical pathology related to vitamin E deficiency?

Semi-Starvation. "The pigmentation of the skin in the semi-starved individual has some resemblance to the pigmentation of Addison's disease."⁶⁷ The semi-starved person burns up fatty stores in the absence of adequate calories; he is often hypersensitive and eventually becomes lethargic. Research reveals that histochemical changes occur in the nervous system in experimental starvation.⁶⁸

Addison's Disease. "Brown pigmentation of the skin, particularly over the knuckles and other joints, is often the first sign observed (in Addison's disease). The skin may first appear tanned or bronzed and then become muddy, brown and unwashed in appearance."⁶⁹ There is often weight loss; and symptoms that reflect nervous involvement such as, amnesia, gastrointestinal discomfort, diarrhea, tension, irritability and depression. Addison's disease is a condition in which there is a hypofunction of the adrenal cortex which normally produces cortical steroids. There is an increased amount of MSH (melanin stimulating hormone) in the urine and blood of these patients. Melanin and adrenalin are derived from the same amino acid, tyrosine.

⁶⁷E. W. McHenry, "Nutrition and Endocrine Function," Borden's Review of Nutrition Research, XVI:2:20.

⁶⁸Z. G. Tsagareli, "Structural and Histochemical Alteration of the Nervous System as a Result of Hunger," Tr. Inst. Eksperim. Morfol., Akad. Nauk Gruz. SSR 11, 51-59, 1963, (Russian), as cited in Chemical Abstracts, Vol. 63, #977f, 1965.

⁶⁹Kathleen N. Shafer, Janet R. Sawyer, Audrey M. McCluskey, and Edna L. Beck, Medical-Surgical Nursing, Third edition (St. Louis, Mo.: The C. V. Mosby Company, 1964), p. 647.

Schizophrenia. Cooley states that adrenochrome is a breakdown product of adrenalin and that small amounts injected into normal individuals produce hallucinations similar to LSD.⁷⁰ Lea reports the results of his research:

In view of reports in the literature suggesting that schizophrenia is essentially a disorder of metabolism possibly related to adrenochrome, the author studied characteristics of schizophrenia in two control series (5, 127 cases) and reports excess of dark hair in schizophrenics in the age group 15-19 years....It is suggested that the dark pigmentation is linked to hepatic dysfunction, both hereditary and acquired, which in turn causes excess of adrenochrome production....adrenalin is derived from tyrosine and that tyrosine is also a precursor of the normal biochrome melanin..."⁷¹

If there is an excess production of adrenochrome (a break-down product of adrenalin in schizophrenia, and adrenalin and melanin are derived from the same amino acid; is there, then, a chemical relationship between excess MSH (melanin stimulating hormone) production, Addison's disease and schizophrenia? Could this explain the schizoid-like behavior of pregnant women that Caplan describes?⁷² Pregnant women have increased MSH in their urine, (page 34).

B-thalassemia Hemoglobin E Disease in Thailand. This disease has an increase in lipochrome pigment in visceral organs and smooth muscle. There is often a hereditary anemia with an hyperplasia of the hematopoietic tissue.

⁷⁰Donald G. Cooley, Modern Medicines (New York: Pocket Books, Inc., A Giant Cardinal Edition, 1963), p. 146.

⁷¹A. J. Lea, "Investigation of Adrenochrome as a Cause of Schizophrenia," Journal of Mental Science, 101:538-547, June, 1955, (Abstract #716, What's New, No. 193, Winter, 1956, Abbott Lab., Chicago, Illinois.)

⁷²Gerald Caplan, An Approach to Community Mental Health (New York: Grune and Stratton, Publishers, 381 Fourth Ave., 1961), pp. 72-36.

Cirrhosis of the liver sometimes accompanies the disease.⁷³ (Anemia is a symptom of vitamin E deficiency as well as the lipochrome pigment in visceral organs and smooth muscle.)

Neiman-Pick Disease. This disease is characterized by lipid degeneration of bodily organs, including the brain, and occurs chiefly among Jews (hereditary factor). There is liver and spleen involvement, anemia and a brown discoloration of the skin. Blindness occurs later.⁷⁴

Kwashiorkor's Anemia. This protein deficiency disease has characteristic changes in pigmentation of the skin and hair. Oski indicates it might be related to vitamin E deficiency. Research relative to this is controversial with some researchers saying there is a relationship and others denying it.

Retinitis Pigmentosa. Oski also indicates that there is a retinitis related to vitamin E deficiency. This disease is frequently hereditary, there is retinal sclerosis with pigmentation and atrophy. There are star shaped deposits of pigment in the retina.⁷⁵ Lerner calls attention to early investigations relative to the use of pituitary intermediate cell extracts for eye disorders and the need to repeat these using pure hormones rather than the extracts, since the early extracts are a mixture of ACTH, a-MSH and B-MSH. The individual affects

⁷³Bhamarapravati, Natth, Sup Na-NaKorn, Prawase Wasi and Sood-sa korn Tuchinda, "Pathology of Abnormal Hemoglobin Diseases Seen in Thailand," The American Journal of Clinical Pathology, 47:6:745-759, 1967.

⁷⁴Helen C. Latham, and Robert V. Heckel, with the collaboration of Margaret Moore, Pediatric Nursing (St. Louis, Mo.: The C. V. Mosby Company, 1967), p. 276.

⁷⁵Dorland's Medical Dictionary, loc. cit.

have not been differentiated.⁷⁶

Von Recklinghausen's Disease (Neurofibromatosis). In this condition a skin pigmentation is diagnostic and may be present at birth: convulsions and mental retardation are presenting symptoms. This syndrome is one of a group of neurocutaneous syndromes.⁷⁷

Urticaria Pigmentosa (X-anthelasmaidea). This condition is characterized by hive-like pigmented macules, modules or maculopapules and urtication results when the brownish or yellowish lesion is rubbed.⁷⁸

Summary of Conditions with Pigmentation. The common denominator in the above conditions and diseases is the presence of a characteristic pigmentation among the symptoms listed as being a part of the pathological picture. The involvement of nervous and epithelial tissues seems to be repetitious. Liver disease is mentioned in several of the conditions, as well as ocular involvement. Nutrition is a factor in three of the conditions. Several of the diseases appear to be influenced by hereditary factors. Anemia is repetitious. The frequent mention of lipid metabolism appears to link this factor to the irregular pigmentations. The possible chemical relationship of the MSH (melanin stimulating hormone) production in pregnant women, Addison's disease and the adrenochrome production of schizophrenia need to be investigated to see if there is any relationship.

⁷⁶Lerner, op. cit., pp. 59-62.

⁷⁷Elena Boder, Current Pediatrics Therapy, editors, Sidney S. Gellis and Benjamin M. Kagan. (Philadelphia, Pa.: Saunders, 1966-67), pp. 75-76.

⁷⁸Andrew M. Margilethe, Current Pediatrics Therapy, Ibid., p. 556.

F. A Summary of the Findings Relative to Pigmentation in Literature

Melanin is a normal pigment in the skin influenced by the pituitary gland and originating from the same amino acid from which adrenalin is derived. It evokes dramatic responses in melanocytes which are nerve cells. Melanin can promote the non-enzymic oxidation of NADH (the reduced form of nicotinamide adenine dinucleotide) to NAD (nicotinamide adenine dinucleotide). (The reduced melanin can be subsequently autooxidized producing melanin and hydrogen peroxide.) This process was speeded up in the presence of light. Hydrogen peroxide and the thiols inactivate and reactivate MSH producing an intricate balance of chemicals that interact as a homeostatic mechanism.

Injections of MSH (melanin stimulating hormone) cause the skin to darken in twenty-four hours and sex hormones appear to sensitize the skin to MSH action.

MSH activity is increased in persons with adrenocortical insufficiency; and the amount of MSH in the urine increases during pregnancy. MSH does influence skin pigmentation and may effect fat metabolism and the nerve synapses.

Horwitt discusses a tissue pigmentation that is apparently due to oxidation of polyunsaturated fats. This pigment is a brownish, acid fast pigment that is indistinguishable from the "ceroid" pigment isolated from livers of rats with nutritional cirrhosis. This PAS positive pigment that is associated with vitamin E deficiency is transformed morphologically in the course of aging, in parallel with oxidation and polymerization of the lipids deposited. These "cell clinkers" are formed by the remains of lysosomes which attract and hold enzyme stain. They are the result of the oxidation of cell components, which are

composed mainly of lipoproteins, in the absence of antioxidants of which vitamin E is one.

The lipofuscin or age pigment is deposited in various tissues as a person grows older and the normal rate of deposition in tissues gives a clue to a person's age. However, stress can increase the rate at which the deposition of pigment occurs. Selye indicates that vitamin E can prevent premature aging due to stressors.

Vitamin E deficiency produces a brown pigmentation in various tissues in the human as well as in animals. The rectal biopsy was suggested as a means of diagnosing pathological tocopherol deficiency. However, the brown bowel did not appear until after three years on a vitamin E deficient diet in two human subjects.

In conditions in which pigmentation is a prominent symptom there appears to be an interrelationship between faulty lipid metabolism, anemias, liver disease, ocular involvement, and pathology involving nervous and epithelial tissues seem repetitious. Several diseases had hereditary factors. Poor nutrition appeared to be a factor in some.

Some Questions. Could there be an enzyme that varies genetically so that certain family groups have problems of fat metabolism and pigmentation; could its absence indicate an increased need for antioxidants? Why do patients with Addison's disease and pregnant women have more MSH (melanin stimulating hormone) in their urine?

Chloasma gravidarum is a recognizable phenomenon, a blotchy pigmentation associated with pregnancy. Can significance be attached to it as a

possible symptom? Is the pigment of chloasma gravidarum the same as lipofuscin, or ceroid material? or PAS-positive material? Does the increased amount of melanin produced during pregnancy create a need for an increased amount of antioxidant? Can this phenomenon be considered a warning of pending chemical imbalance? Why is it seen only in some pregnant women? If damage does occur due to peroxidation, is it reversible? Does peroxidation influence the fetus and the new born infant?

CHAPTER V

VITAMIN E DEFICIENCY, INCREASED MELANIN PRODUCTION AND REPRODUCTION

The purpose of this chapter is to focus directly on vitamin E deficiency and its influence on the reproductive system, the fetus and the infant, and to consider the possible influence of increased melanin production during pregnancy.

A. The Effect of Vitamin E Deficiency on Reproduction

Endocrine Changes of Pregnancy and Stress. The period of pregnancy and gestation are considered to be a period of additional stress in the life of a woman. One of the reasons for this stress is the physiological changes of pregnancy that include (1) the development of the placenta --an organ of internal secretion, forming in woman chorionic gonadotropin, oestrogen, and progesterone, (2) increase in the size of the pregnant uterus, relaxation of the pelvic ligaments, and enlargement of the birth canal, (3) formation, persistence, and degeneration of the corpora lutea, and cessation of ovulation and menstruation, and (4) development of the breasts and the onset of lactation.⁷⁹ Pregnancy is characterized by instability in many women as they experience hormonal changes.

There is a period of adjustment following pregnancy that is often characterized by the phrase, "postpartum blues." In a study involving a sample of one-hundred thirty pregnant women, the stresses of the child bearing year were analyzed. The author concluded that "the early postpartum weeks and months generated the greatest number of stresses, and the hospital stay, the fewest."

⁷⁹Keele, op. cit., p. 527.

Among the stresses listed as occurring at various stages or segments of the child bearing year, the emotional upsets, fatigue and depression were repetitious. "While none of the one-hundred thirty mothers...had been recognized as having psychiatric illness, several vividly described their despair and exhaustion."⁸⁰

MSH Increase During Pregnancy. The pituitary gland enlarges during pregnancy. It is thought that the increase of MSH (melanin stimulating hormone) during pregnancy is due to this enlargement. With the increased amount of MSH there is increased pigmentation due to melanin. This increased pigmentation affects the pregnant woman in several ways, e. g., the darkening of the nipples and the linea nigra. For some women chloasma gravidarum is a manifestation and for most it disappears following pregnancy. According to the research of VanWoert (pages 32-33), this melanin can promote a chain reaction that produces hydrogen peroxide in the cell and, therefore, this investigator concludes that the need for an antioxidant is increased. If biological antioxidants are absent, could cellular tissue and, thus, organ and system damage progress until a balance of pro-oxidants and antioxidants is reached?

Lerner indicates the influence of hydrogen peroxide on the production of more melanin and hydrogen peroxide. Is the careful balance more easily upset in pregnancy due to hormonal changes?

The Damage of Vitamin E Deficiency on the Reproductive System. The first experiments done that demonstrated vitamin E deficiency were done

⁸⁰Virginia L. Larsen, "Stresses of the Child Bearing Year," American Journal of Public Health, January, 1966, p. 32, abstracted in Briefs, April, 1966.

relative to the reproductive system by Evans, et al., in the early 1920's. A reading of these experiments is revealing, even today. They demonstrated that, in the absence of vitamin E, male rats could be made sterile and that the female rat is more prone to miscarriages. If E deficiency was severe, fetal resorption occurred. Since then many persons have verified the findings of Evans, et al. According to Keele, "In the female vitamin E deficient rat, oestrus, ovulation, coitus, and implantation of the ovum in the uterus occur normally, followed by the death of the fetus." And Evans has stated that prolonged deficiency may make recovery from vitamin E deficiency incomplete; however, as he has demonstrated, a degree of recovery is possible with the inclusion of vitamin E in the diet.

Multiple Variables Produce Varying Results. Variation in the results of many researchers has been confusing. The multiplicity of the existing variables related to vitamin E deficiency have contributed to this confusion. The length of time necessary to produce deficiency varies with such factors as: 1) the amount of polyunsaturated fat intake, 2) the lipid stores in the individual, 3) the susceptibility of the subject to deficiency due to heredity, 4) the length of time necessary for the individual to become vitamin E depleted, and 5) the time required to bring vitamin E serum levels back to normal. The time component is longer than has been believed by some researchers. This variable may account for the varied results of researchers.

Specificity for the Reproductive System. Horwitt states that "much has been made of the specificity of the action of vitamin E with regard to testicular degeneration in the male rat." This is better understood if interpreted along with the observation that "even on a low fat diet in which the need for a-tocopherol

by other tissues is very low, testes of rats have high levels of polyunsaturated fatty acids. " Thus, the level of polyunsaturated fat in the testes contributes to the early manifestation of vitamin E deficiency in relation to the reproductive system in the male. In the female, increased MSH (melanin stimulating hormone) production and its chain reaction which depletes the stores of antioxidants could contribute to the specificity for the reproductive period.

Lactation is Influenced. According to Horwitt, during lactation, changes in fatty acid distribution can take place depending upon the stores and diet of the mother, because polyunsaturated fat of milk lipids can be modified by diet.

B. The Effect of Vitamin E Deficiency on the Fetus and on Infants

Hemolytic Anemia in Premature Infants. In 1967 Oski and Barness demonstrated that the hemolytic anemia that often occurs in premature infants with low serum tocopherol levels responds to vitamin E therapy. At six weeks of age these infants had high reticulocyte counts in the presence of a falling hemoglobin and a large number of irregularly contracted erythrocytes similar to the cells of patients with acanthocytosis, and that such patients were markedly vitamin E deficient. Some of these infants had bone marrow changes and shortened red cell survival but had not developed creatinuria. Because of the absence of creatinuria, it was stated that "the disease...may be a manifestation of mild vitamin E deficiency superimposed on an unusual fatty acid composition of the red cell membrane."⁸¹

⁸¹Oski, loc. cit.

Some Symptoms of Vitamin E Deficiency in Infants. Binder and Spiro state that the "intake of polyunsaturated fatty acids without added tocopherol (in infants) leads to a group of symptoms and signs characterized by irritability, pitting edema, and a variety of skin changes. They were all corrected by vitamin E supplementation."

Horwitt reports a case of cerebellitis in a fourteen month old infant who had vitamin E deficiency. Mattill states that massive necrosis of the liver can be produced in young rats by vitamin E deprivation.⁸² WeiKing's experiment indicated that "prenatal vitamin E deficiency affected the normal chemical development of the embryonic livers" of rats (page 22).

Effects on the Fetus. As stated above there is liver damage in the fetuses that are developed on prenatal vitamin E deficient diets. Horwitt indicates that there is "difficulty for small concentrations of tocopherol...(to) cross the placenta." In regard to encephalomalacia, he states that "studies of teratogeny have included reports of neurological changes in the rat fetus being developed on a low vitamin E regimen," and that chicks hatched from the eggs of vitamin E deficient hens are more susceptible to encephalomalacia.

The diet of the mother can affect the fetus. Horwitt states, "the polyunsaturated fatty acids of the lipoprotein or mitochondrial lipid in a fetus can vary with the lipid stores of the mother, which...could be more a function of the past than present diet,...and that the lipids from brain and liver mitochondria can be varied more than 300 per cent by dietary means."

⁸²Henry A. Mattill, "Vitamin E--The Tocopherols," Borden's Review of Nutrition Research, XIII:9, December, 1952.

Horwitt recommends that "the studies of retrolental fibroplasia in pre-mature infants in regard to severe decreases in erythrocyte survival time" need to be reinvestigated in the light of newer knowledge related to vitamin E deficiency and levels of polyunsaturated fatty acids.

How much influence does vitamin E deficiency have on the pathological conditions of the maternity patient and the infant she is bearing? Is chloasma gravidarum just a passing phenomenon?

C. Caplan's Observation of Schizoid-Like Behavior in Pregnant Women.

The frequent mention of brain and nerve damage with pigment deposits in vitamin E deficient animals, as well as the case of cerebellitis in a fourteen month old infant that Horwitt observed, calls attention to the possibility that nervousness and mental illness might be more prevalent in persons who are vitamin E deficient and in persons with increased amounts of MSH (melanin stimulating hormone).

In discussing the psychology of pregnancy, Caplan calls attention to the mood swings that seem to be connected to metabolic alterations of pregnancy that begin at the end of the first trimester and continue two to three weeks post-partum. He observed that pregnant women often fantasize and openly express some of these fantasies with each other in prenatal classes; that they are freer to speak of repressed material that emerges into consciousness during counseling sessions. Often they become preoccupied with early conflicts. Later, following pregnancy, they no longer remember that they even spoke of these fantasies or repressed ideas and are embarrassed to express them. While these women do not seem to have a break with reality, yet they experience a period of

heightened sensitivity and lessened emotional stability.⁸³

It is thought that schizophrenics have increased amounts of adrenochrome which is derived from tyrosine which also is a precursor of melanin (page 33, and Figure 1, page 58). Wooley states that a serotonin synergist has been found in schizophrenics and that there is a serotonin deficiency in phenylketonuria patients.⁸⁴ According to Cooley there is evidence of a biochemical factor that appears to interfere with appropriate release of energies.

Somehow involved with thought, behavior, and chemical transmissions between nerves are potent "brain amines" such as serotonin, epinephrine (adrenalin), and norepinephrine. These nitrogenous constituents of the nervous system are derived from amino acids of proteins, are perfectly "natural," and appear to be released in small amounts all the time. There are striking chemical resemblances between some natural brain amines and some hallucinogenic drugs that produce remarkable imitations of schizophrenia....Mental disturbances conceivably might result from infinitesimal amounts of mescaline-like toxins made by mistake from precursors of the adrenal hormone.

Adrenochrome, a breakdown product of epinephrine, plays havoc with the brain when minute amounts are injected. LSD-25 has a chemical resemblance to mescaline. Various amines that produce hallucinations are highly reactive compounds that seem capable of changing from one to another rather easily.⁸⁵

The increased MSH (melanin stimulating hormone) production of pregnancy seems linked to the "brain amines," and melanin production. Excessive pigmentation may be the symptom that forewarns of a pending chemical imbalance that can possibly produce the schizoid-like behavior that Caplan describes.

There may be an interrelationship between: 1) the schizoid-like

⁸³Gerald Caplan, An Approach to Community Mental Health (381 4th Ave., New York, 16, N.Y.: Grune and Stratton Publishers, 1961), pp. 72-86.

⁸⁴D. Wayne Wooley, Neurophysiology and Emotion, edited by David C. Glass (New York: The Rockefeller University Press and the Russel Sage Foundation, 1967), pp. 108-117.

⁸⁵Cooley, op. cit., pp. 147-148.

behavior of pregnant women; 2) adrenalin and melanin are derived from the same amino acid (tyrosine); 3) pregnancy is considered to be a stressor period of life; 4) the presence of adrenochrome (a break-down product of adrenalin) in schizophrenia; 5) Addison's disease and MSH (melanin stimulating hormone) in the blood and urine; 6) pregnancy and increased MSH in the urine; 7) pigmentation as a symptom of Addison's disease; 8) an additional need for vitamin E at times as an antioxidant; and 9) chloasma gravidarum, the blotchy brown pigmentation of the face that sometimes occurs in pregnant women.

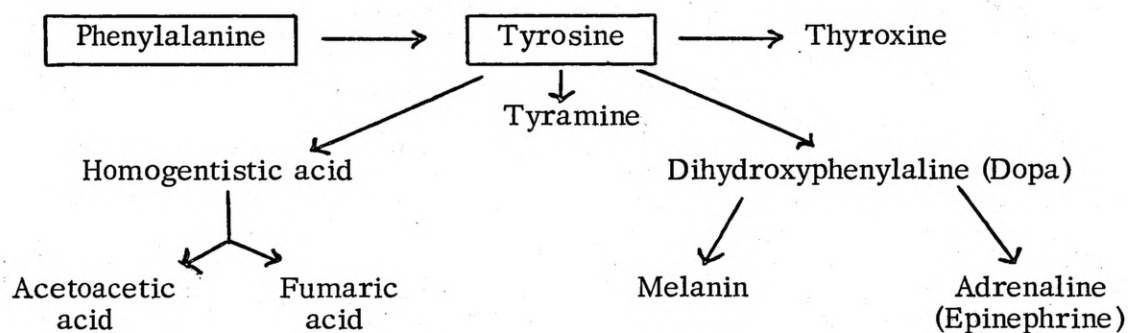


FIGURE 1

METABOLIC PATHWAYS OF PHENYLALANINE AND TYROSINE⁸⁶

⁸⁶Abraham Cantrow, and Bernard Schepartz, Biochemistry (Philadelphia: W. B. Saunders Company, 1954), p. 549.

D. Summary

The endocrine changes of pregnancy are accompanied by emotional instability and stress during the child bearing year. This may be in part due to increased melanin production as a result of increased MSH (melanin stimulating hormone) that may increase the need for an antioxidant to maintain a chemical balance. One of the functions of vitamin E is to serve as an antioxidant. Vitamin E does influence reproduction, the fetus and the newborn infant. The specificity for the reproductive and nervous systems seems to be related to the high content of polyunsaturated fats of these systems. This is also a factor in red blood cell damage. The schizoid-like behavior of pregnant women may be related to the phenomenon of chloasma gravidarum.

CHAPTER VI

A BROAD VIEW OF VITAMIN E DEFICIENCY AND PIGMENTATION

Following the summarization of the functions of vitamin E and depicting of the emerging pattern of tocopherol deficiency, some consideration needs to be given to the requirements and nutritional availability of vitamin E. Literature reveals some interesting uses for vitamin E. This chapter will conclude with a summarization of the literature review relative to vitamin E deficiency and pigmentation.

A. A Summarization of the Functions of Vitamin E

Biologically the functions of vitamin E include: 1) antisterility and progestational; 2) antianemic; 3) antioxidant and as a neutralizer of free radicals; 4) in the development and maintenance of muscle integrity; and 5) a repressor of the lysosomal enzymes that are catobolic (Chapter III, B).

In addition, it is thought to protect vitamin A and results in influencing the reproductive system, epithelial tissues, mucous secretions, and photo-reception.

These functions are at the cellular level and involve biochemical changes.

B. The Emerging Clinical Picture of Vitamin E Deficiency

According to Binder and Spiro the one common denominator of vitamin E deficiency is steatorrhea, (excessive loss of fat in the feces). "It is apparent that laboratory evidence of tocopherol deficiency is not equivalent to biological tocopherol deficiency...Tocopherol deficiency, presumably secondary to

steatorrhea, has been demonstrated." The duration of the condition does appear to be important for the occurrence of more severe tissue depletion to occur. The malabsorption of fats can persist for at least six to twelve months, even though the subject is again supplied with an adequate dosage of vitamin E.

Other factors. Other factors, that may have a bearing on the clinical picture of tocopherol deficiency include: the possibility of heredity predisposition; the level of polyunsaturated fat intake; the polyunsaturated content of various tissues; the possibility of increased need for an antioxidant during stressor periods of life as evidenced by the deposition of "lipofuscin" or PAS-positive material; and in relation to increased production of melanin, which may promote additional need for antioxidants.

Symptoms. Harris, in a discussion relative to the papers presented at a Nutrition Symposium, attempted to depict the symptoms of tocopherol deficiency. The first symptom of deficiency in adults was RBC (erythrocyte) hemolysis with an increased pulse rate. Muscle degeneration with creatinurea is the next possible manifestation. Ceroid or PAS deposition in smooth muscle will occur along with creatinurea. In non-thriving premature or small infants, that develop hemolytic anemia at six to ten weeks, tocopherol deficiency should be considered as a possibility. Creatinurea did not occur in the infants Oski and Barness observed. They indicated that perhaps it was because this symptom is one of a more advanced deficiency. Irritability, edema and skin lesions in addition to anemia in infants might be indicative of tocopherol deficiency.

That focal necrosis occurs in vitamin E deficiency has been stated by more than one researcher. The tissues that become diseased due to tocopherol deficiency and subsequently damaged, will reflect the specific symptoms related

to the effected tissue; e. g., reproductive, endocrine, circulatory, nervous system, etc.

Diagnostic Procedures. It might be of value for nurses to know of some of the diagnostic procedures that could be used in determining if vitamin E deficiency is a health problem. While these procedures are not usually done in ordinary clinics or hospital laboratories, they have been used in experimental laboratories. Some of these are: 1) the hydrogen peroxide induced hemolysis test;⁸⁷ 2) assay of tocopherol plasma levels (Quaife and Dju method);⁸⁸ the Rose-Gyorgy Erythrocyte Hemolysis Test;⁸⁹ tissue biopsy and analysis of the tissue for PAS positive material;⁹⁰ and others are listed in literature and laboratory manuals.

The fact that laboratory evidence of tocopherol deficiency is not equivalent to biological tocopherol deficiency indicates the importance of close observation and accurate recording of all possible symptoms. In the light of the findings of many researchers of unusual pigmentation in various tissues of subjects with vitamin E deficiency, the recording of unusual skin pigmentation may aid the doctor in making a diagnosis; it may be of value to researchers who attempt to collect data from charts.

⁸⁷P. J. Leonare, and M. S. Losowsky, "Relationship Between Plasma Vitamin E Level and Peroxide Hemolysis Test in Human Subjects," The American Journal of Clinical Nutrition, 20:8:795-799, August, 1967.

⁸⁸Philip B. Hawk, and Bernard L. Oser, and Wm. H. Summersen, Practical Physiological Chemistry, 13th edition (New York: The Blakiston Company, Inc., 1954), p. 1273.

⁸⁹Ibid., pp. 1275-1276.

⁹⁰Binder and Spiro, op. cit., p. 598.

C. Consideration of Requirements of Tocopherol

Standard Requirements. In 1967, Harris, director of the Division of Nutrition, Bureau of Science, Food and Drug Administration, Washington, D. C., stated that the estimated adult requirement for a-tocopherol is about ten to thirty IU (international units) per day (depending upon the amount of polyunsaturated fatty acids that are in the diet and in the tissues). This estimate remains unchanged following Dr. Horwitt's Elgin experiments on humans.⁹¹ In 1969 the dosage is quoted as unknown.⁹²

The requirement level for infants is based on the content that is usually available in human milk. Horwitt states that this needs to be reevaluated in relationship to PUFA (polyunsaturated fatty acid) intake. The possibility that human milk could reflect a deficiency in the mother concerns this writer. Infant requirements were previously set at 0.5 mg. a-tocopherol per kg. of body weight per day. Horwitt thinks this may be too low. The therapeutic doses used for infants by Oski was 15.7 mg. in aqua-sol or d-alph-tocopherol 9 mg. per day.

An international unit is based on the amount necessary to prevent sterility in the rat. 1 mg. of DL-a-tocopherol acetate is equal to one IU (international unit) of vitamin E.⁹³

Varied Requirements. Persons who absorb fats poorly may eventually

⁹¹Harris, op. cit., p. 602.

⁹²Andres Goth, Medical Pharmacology Principles and Concepts, 4th edition (St. Louis, Mo.: C. V. Mosby, 1968), p. 580.

⁹³Hawk, op. cit., p. 1274.

develop vitamin E deficiency symptoms. Harris states that only about forty to sixty per cent of tocopherol is absorbed from the G.I. tract. Malabsorption syndromes decrease that percentage. He asks the question: (Do) "diets containing less than 0.6 mg. of alpha-tocopherol to 1.0 mg. of polyunsaturated fatty acids (an E/PUFA ratio of less than 0.6) deplete the organism of vitamin E, apply generally to human beings? Clinical trials so far have been both positive and negative--more studies are needed, preferably long-term ones with well controlled diets."

In his review Horwitt states that

the need for vitamin E will also vary with the changes in the polyunsaturated fatty acid profiles of the tissue lipids (and)...the feeding of additional polyunsaturated fatty acids containing lipids. With these variables it is not possible to base requirements on the usual concepts of species, state of growth and size. When the PUFA intake is only sufficient to supply minimal needs for health, very little vitamin E seems to be required. When the ingestion of PUFA is high for prolonged periods, ...the tocopherol requirement increases many fold...The levels of plasma tocopherol adjust more slowly than originally thought. In a case in which there was prolonged mild deficiency with increased PUFA intake, as much as 60 mg. of d-a-tocopherol per day for many months was barely adequate to return plasma tocopherol... to a point where it would remain normal upon removal of the supplementation.

Selye's experiment demonstrated the need for additional vitamin E during periods of stress. Pregnancy, as a stressor period, might influence the requirement for vitamin E.

Reversal of Pathology. According to Horwitt, reversal of pathology is not always possible and when it is possible it is a long slow process. Also, it is possible to have pathology with creatinurea for periods of time before pathology manifests itself in other ways, for "it is possible for the erythropoietic system to make up the difference for a period of time."

Tocopherol Substitutes. Tocopherol substitution by other products has been controversial. Horwitt states that

It is probably fair to say that no complete, non-toxic substitute for a-tocopherol has been found. Not only do the other tocopherols not fill the bill in all animal tissues, but to date all the synthetic tocopherols have been shown, in one way or another, to produce different physiological effects when used. At least some of the synthetic antioxidants differ from a-tocopherol in the manner in which they transfer across cell walls (and the placenta), and accumulate in desirable concentration where needed.⁹⁴

D. The Nutritional Availability of Vitamin E

Sources of Vitamin E. Proudfit states that the oils of wheat germ, cottonseed, rice germ, and the germs of other seeds are potent sources of vitamin E and that green leafy vegetables, nuts and legumes have some.⁹⁵

Horwitt indicates that meat and eggs contain some, if the animals have an adequate supply of vitamin E. Foods that are high in vitamin E content are often, also, high in PUFA. Food products reflect the tocopherol content of the soil on which they grow.

Milk. The vitamin E content of cows milk is inferior to human milk and the new born infant may be shorted on vitamin E if he is on an unsupplemented formula.⁹⁶

Processing Effects Tocopherol Content. According to Horwitt "commercial and cooking processes tend to destroy the natural balance of PUFA

⁹⁴Horwitt, op. cit., pp. 3, 10.

⁹⁵Fairfax T. Proudfit, and revised by Corinne H. Robinson, Normal and Therapeutic Nutrition, 12th edition (New York: The Macmillan Company, 1961), p. 127.

⁹⁶Harris, op. cit., p. 602.

(polyunsaturated fatty acids) to tocopherol in many foods." Some experiments indicate that heat does not destroy vitamin E but others indicate heating does destroy the vitamin E content of foods.⁹⁷ Irradiation of cereals for pests did not show immediate loss of the tocopherol levels but there is subsequent deterioration of the cereals in storage.⁹⁸

A recent analysis of infant formulas and cereals revealed that at least one brand had low levels indicating much destruction of tocopherol during certain processing of cereals for infant consumption. "These infant formulas had such low levels of alpha-tocopherol that they might be unsatisfactory sources of tocopherol for infant consumption. Supplementation of all infant formulas with alpha-tocopherol is indicated."⁹⁹

Rancidity. Rancidity of oils tends to destroy the tocopherol.¹⁰⁰ Swine products are more stable from animals that have had tocopherol supplements.¹⁰¹

⁹⁷H. E. Schmidt, "Determination of Tocopherols in Oils and Fats: Influence of the Tocopherols Content of Groundnut and Soybean Oils on the Course of Oxidation of These Oils by Heating," (Univ. Forschung Slab., Hamberg, Germany), Fette, Seifen, Anstrichm. 69 (12), 912-16 (1967), (Ger.), Chemical Abstract 48421u.

⁹⁸Jan Wozniak, "Effect of Ionizing Radiation on Pests in Cereals," (Panstiv. Zahl. Hig., Warsaw), Roczniki Panstrvowego Zakladn Hig., 16 (2), 183-8 (1965), (Pol.); cf. Chemical Abstract 63 13931c.

⁹⁹Dicks-Bushnell, Martha W., and Karen C. Davis, "Vitamin E Content of Infant Formulas and Cereals," The American Journal of Clinical Nutrition, 20:3:262-269, March, 1967.

¹⁰⁰W. A. Krehl, and J. J. Barboriak, "Factors Affecting the Utilization of Food Nutrients by the Normal Organism," (Chapter 13), Nutritional Evaluation of Food Processing, edited by Robert S. Harris, and S. B. Loesecke (New York, London: John Wiley and Sons, Inc., 1960), p. 533.

¹⁰¹R. Grau, "Effect of Tocopherol Supplementation on the Quality of Meat Products," (Bundesanstalt Fleischforsch., Kelkheim, Germ.), Wiss. Veroeff. Deut. Ges Erache. 16, 198-98 (1967) (Germ), Chemical Abstract 10749u, p. 1017.

There needs to be continued research to clarify the most stable sources and methods of processing that maintain the proper balance of tocopherol to polyunsaturated fatty acids in the diet.

E. Varied Uses of Tocopherol

New uses have been found for tocopherol that are not necessarily related to its deficiency. The shelf life of stored blood was extended by the addition of a-tocopherol acetate.¹⁰² The tocopherol pretreatment of subjects on the heart-lung machines significantly reduced their hemolytic effects.¹⁰³ Its anti-inflammatory activity in relation to linoleic acid may be a possible use.¹⁰⁴ Other uses are suggested in literature and being studied in research laboratories.

F. A Summary of the Literature Survey

Vitamin E deficiency is now known among researchers to occur at the intracellular level; its absence causes definite cell damage by the interaction of the unneutralized end products of cell metabolism or the chemical imbalance that requires antioxidation of oxidizing molecules. It is possible for the deficiency to effect all types of cells, tissues, organs and systems including the reproductive system in both animals and humans. However, this deficiency is

¹⁰²Howard D. Stowe, "Alpha-Tocopherol Requirements for Equine Erythrocyte Stability," The American Journal of Clinical Nutrition, 21:2:135, February, 1968.

¹⁰³Ibid.

¹⁰⁴V. Wilford Stuyvesant, and Weldon B. Jolley, "Anti-inflammatory Activity of d-a-tocopherol and Linoleic Acid," (School of Med., Loma Linda Univ., Loma Linda, Calif.), Nature, 216 (5115), 585-586 (1967), Chemistry Abstract, Vol. 68, 11566a.

often selective as it effects the cellular parts and tissues that have a higher concentration of polyunsaturated fat. When biological antioxidants and cellular lipids are out of balance, excessive oxidation of cellular lipoproteins occurs.

Vitamin E can be prevalent in the diet and the person still be deficient if that person follows a diet high in polyunsaturated fats; or if that person has a special problem (there seems to be an hereditary tendency) with the absorption and utilization of fats thus excreting a higher proportion of the fat in the feces along with the fat soluble vitamin E.

A brown discoloration of various tissues has been noted by researchers in subjects with vitamin E deficiency. Brown pigmentation of the lower bowel will occur, but it may be delayed for about three years after vitamin E depletion in some persons. If biopsied the specimens reveal ceroid deposits. The process of aging as indicated by the degree of pigmentation in cells and tissues, and is speeded up in the absence of antioxidants (vitamin E is one). Increased production of melanin promotes the nonenzymic oxidation of intermediates; the chain reaction leads to the production of hydrogen peroxide and more melanin which in turn activate and inactivate MSH (melanin stimulating hormone).

The first symptoms of vitamin E deficiency to appear in the human are the high fat content of the feces and hemolysis or fragility of the red blood cell with an increased pulse rate. Eventual tissue damage will produce creatinurea. Pigmentation of various cells and tissues will occur; though, when it will occur and the extent or degree are still not determined. Other symptoms are related to the specific tissue or tissues involved in the deficiency.

Providing the cells with an adequate amount of vitamin E (a normal constituent of cells), to neutralize the end products of cell metabolism and

reducing the level of polyunsaturated fats, aids in bringing cellular balance and thus preventing further damage. How much rejuvenation of tissues is possible (some is) is still not determined. Prolonged deficiency produces permanent damage.

Foods that are high in vitamin E content are also high in polyunsaturated fats. The level of vitamin in plants and animals reflect the source. Rancidity, and processing affects the level of vitamin E in foods with some processed infants' foods being considered as an inadequate source of tocopherol unless supplemented.

Alpha-tocopherol is the most significant form of vitamin E. Experiments reveal that the availability of synthetic tocopherol to cross cell membranes is not the same as in the natural products.

Present standards do not indicate a recommended daily requirement as they are under revision in light of new research. Research reveals a wide range in the need for vitamin E with many variables affecting it. Some of these are: the level of polyunsaturated fats in the diet; the hereditary tendency; the length of time the person has been deficient; additional stress by such stressors as increased production of melanin. Some persons need a daily intake of 60 mg.; especially, if they have previously been depleted. Therapeutic doses go as high as 200 to 300 mg. Dr. Selye's experiment with rats used a dosage of 1,000 mg. (I.U.) in the treated rats that recovered.

Among the purposes of this literature survey is to delineate, if possible, the pattern of a vitamin E deficiency syndrome as revealed in literature. The pattern is emerging.

Research is being done relative to melanin and its effects in animals and

in humans. Melanin is a normal pigment in the skin, hair, and choroid coat of the eye. Melanocytes are nerve cells and MSH (melanin stimulating hormone) evokes dramatic responses in them. MSH is produced by the pituitary intermediate cells and injections can produce a skin darkening. MSH activity is increased in the blood and urine of patients with adrenocortical insufficiency; and MSH is increased in the urine of pregnant women.

Melanin is produced by the reaction of an oxidase tyrosinase and tyrosine. Tyrosine is the amino-acid that is the precursor of both melanin and adrenaline. Melanin reduces to melanin and hydrogen peroxide. Hydrogen peroxide and the thiols activate and inactivate melanin. Melanin promotes the nonenzymatic oxidation of NADH (the reduced form of nicotinamide adenine dinucleotide) to NAD (nicotinamide adenine dinucleotide), (intermediate molecules of a metabolic process).

a-MSH and B-MSH influence the circulating free fatty acids in rabbits. The E deficiency pigment is morphologically formed in parallel with oxidation and polymerization of the lipids deposited. The age pigment resembles the pigment isolated from vitamin E deficient subjects and contains enzymes of mitochondria and lysosomes.

PART III

A STUDY OF POSTPARTUM PATIENTS WITH CHLOASMA GRAVIDARUM

The literature survey revealed the emerging clinical picture of vitamin E deficiency. Research is being done relative to melanin and its production as well as effects on the human organism. However, very little is known about the "mask of pregnancy" or chloasma gravidarum beyond the assumption that it may be related to adrenal insufficiency. For this reason, and as an outgrowth of open-ended research, data were collected on two small samples of postpartum women with and without chloasma gravidarum. The analysis of the data is included in Chapter VII.

CHAPTER VII

THE ANALYSIS OF DATA COLLECTED ON POSTPARTUM PATIENTS WITH CHLOASMA GRAVIDARUM (AND THEIR INFANTS)

A. Review of Methodology of This Specific Study

The Sample. The "mask of pregnancy" or chloasma gravidarum is used as the identifying characteristic to differentiate between two groups of subjects used for this study. The differentiation of a sample by an identifying characteristic or independent variable can be termed a cohort sample. Those with chloasma gravidarum (fifteen patients) are considered to be the subjects; and those who did not have chloasma (thirty-one patients) are considered to be the control subjects.

Random selection was used by evaluating all the postpartum patients (a total of forty-six patients), who entered the Billings Deaconess Hospital during a three week period from May 5 to May 25, 1968. The new born infants of these mothers are included to evaluate their weight as an index of prematurity.

Tools for the Collection of Data. A five point rating scale (Appendix A) was used to evaluate the degree of chloasma present in all postpartum patients who entered the hospital for obstetrical care. The evaluation was recorded on the Information Collection Form used for each patient (Appendix A). The temperature taking procedure was used as a guise for the investigator's presence when observing patients. The temperatures were taken between 6:30 and 7:30 each morning. The same time of day was used for all observations to eliminate the varying degree of light that might occur between morning and evening and in this way make a difference in the evaluation of the degree of pigmentation present.

The month of May precedes the summer season in which a suntan might heighten the degree of pigmentation known as chloasma gravidarum. In several subjects there was a question of the degree of chloasma present. The investigator elicited some explanation from the patient by asking the subject if she had been out sun bathing. The patients' comments were helpful in clarifying these cases.

Because of the undesirability of calling to the patients' attention, the presence of chloasma gravidarum, information was obtained from charts rather than by direct questioning of the subjects. Similar types of information, that was available on most charts, was transferred to the "Information Collection Form" to be evaluated for similarities and differences.

From the varied pieces of information recorded, eight specific items were chosen for further analysis in relation to the presence or absence of chloasma gravidarum. These were tabulated and null hypotheses formed. The two populations are assumed to have an equal deviation from the means in each hypothesis. The degree of difference between the two populations was tested for the degree of significance by either the chi square or the t-test. The data for the t-tests were processed by computer and a desk calculator was used by the investigator for computing the chi squares. The null hypotheses (N_0) were rejected or not rejected on the basis of these tests of significance of variance.

B. Results of This Study

Of the forty-six postpartum patients who were evaluated for chloasma gravidarum, fifteen manifested a degree of chloasma gravidarum and thirty-one did not show any manifestation of this phenomenon. The degree of chloasma was rated and classified according to a scale. These ratings are shown in Table I.

TABLE I

CLASSIFICATION OF POSTPARTAL PATIENTS WITH AND WITHOUT CHLOASMA

	Without Chloasma	Total With Chloasma	Degree of Chloasma Present			
			Faint	Moderate	Marked	Very Marked
Number of Patients	31	15	7	3	3	2

This sample is too small to indicate that one-third of the general population at large of immediate postpartal patients have chloasma gravidarum to some degree. There might be a sporadic distribution influenced by geography, climate, season, available food supplies and many other variables. The high rate of twins in this sample might influence the results. It is of interest to notice that three of the four sets of twins were born to women who had chloasma.

Hypothesis #1: There is no significant difference in the ages of immediate postpartum women who have chloasma and those who do not have chloasma gravidarum.

This hypothesis was rejected. The age had not been recorded on one subject with chloasma reducing the number of the sample "with chloasma" to fourteen. The t-ratio of 3.40 indicates the difference between the means was sufficiently large enough to reject the null hypothesis at the one per cent confidence level. In other words, this difference between the means would not be expected to occur more than one per cent of the time due to sampling error in the general population of postpartal patients. The results of these findings are

in Table II.

TABLE II

A COMPARISON OF AGE AND THE PRESENCE OR ABSENCE OF CHLOASMA GRAVIDARUM IN POSTPARTUM PATIENTS WHILE HOSPITALIZED

Subjects	N	Mean	S.D.	t-test
With Chloasma	14	27.64	4.98	3.40
Without Chloasma	31	23.07	3.79	There is a significant difference

Hypothesis #2. There is no significant difference in the gravida rank of immediate postpartal women who have chloasma and those who do not have chloasma. (Gravida refers to the ordinal position of the pregnancy.)

This hypothesis was rejected. All of the subjects could be used for the testing of this null hypothesis. The t-ratio of significance indicates that the difference between the means was sufficiently large enough to reject the null hypothesis at the five per cent confidence level. Women who have chloasma gravidarum are more likely to be multiparas. The data and the results of the t-test for this hypothesis is in Table III.

TABLE III

A COMPARISON OF GRAVIDA AND THE PRESENCE OR ABSENCE OF
CHLOASMA GRAVIDARUM IN POSTPARTUM PATIENTS
WHILE HOSPITALIZED

Subjects	N	Mean	S.D.	t-test
With Chloasma	15	3.33	1.89	2.21
Without Chloasma	31	2.35	1.12	There is a significant difference

Hypothesis #3: There is no significant difference in the hematocrit of immediate postpartum women who have chloasma and those who do not have chloasma.

This null hypothesis was not rejected. All of the subjects were used for the testing of this hypothesis. The t-ratio indicates that there was no significant difference in the hematocrit of the two samples. Therefore, the null hypothesis was not rejected. The results and data relative to this hypothesis are in Table IV.

TABLE IV

A COMPARISON OF HEMATOCRIT AND THE PRESENCE OR ABSENCE OF
CHLOASMA GRAVIDARUM IN POSTPARTUM PATIENTS
WHILE HOSPITALIZED

Subjects	N	Mean	S. D.	t-test
With Chloasma	15	38.53	4.54	0.237
Without Chloasma	31	38.23	3.92	The difference is not significant

Hypothesis #4: There is no significant difference in the weight of infants born to women who have chloasma and those who do not have chloasma.

This null hypothesis was not rejected. In testing this hypothesis the presence of three sets of twins in the subjects with chloasma and one set of twins in the control group was enough to distort the results. Because of this, the twins were not included as a part of the samples. The infant weights were used as an index of prematurity.

The t-ratio indicates that the difference between the means was not sufficiently large enough to reject the null hypothesis at the five per cent confidence level. The weights of infants born to mothers with chloasma do not differ significantly from those infants born to mothers without chloasma gravidarum. The results and data are included in Table V.

TABLE V

A COMPARISON OF INFANT WEIGHT (AS AN INDEX OF PREMATURITY)
AND THE PRESENCE OR ABSENCE OF CHLOASMA GRAVIDARUM
IN POSTPARTUM PATIENTS WHILE HOSPITALIZED

Subjects	N	Mean	S.D.	t-test
With Chloasma	12	6.59	0.76	0.59
Without Chloasma	30	6.77	0.96	Difference is not significant

Hypothesis #5: There is no significant difference in the date of delivery--before or after the due date --for women who have chloasma and those who do not have chloasma.

This null hypothesis was not rejected. The due dates were not recorded on the charts for two women for each of the two samples. This reduced the samples from fifteen to thirteen, and thirty-one to twenty-nine. The inclusion of this data was again intended as an index of prematurity.

Though the chi square (X^2) comes close to being significant it was not significant at the five per cent level of confidence, but is significant at the ten per cent level of confidence; that is, the difference between the means could not be expected to occur more than ten per cent of the time in the general population. The birth dates did not vary significantly between those women with chloasma gravidarum. The null hypothesis was not rejected. Table VI contains the data and results relative to hypothesis #5.

TABLE VI

A COMPARISON OF DUE DATE AND THE PRESENCE OR ABSENCE OF
CHLOASMA GRAVIDARUM IN POSTPARTUM PATIENTS
WHILE HOSPITALIZED

Subjects	N	Delivered Before Due Date	Delivered After Due Date	Chi Square
With Chloasma	13	11	2	2.86
Without Chloasma	29	18	11	The difference is not significant

Hypothesis #6: There is no significant difference in the miscarriage rate for women who have chloasma and those who do not have chloasma.

This null hypothesis was not rejected. All of the women could be included in both samples for the testing of this hypothesis. Some women in the sample had more than one miscarriage. For this study they were counted only once. That is, the women were rated as having had a miscarriage or not having a miscarriage, not for the number of miscarriages per woman.

The chi square (X^2) was used to measure the significance of the difference between the two samples. The chi square indicates the difference between the means of the two samples is not significant at the five per cent level. The null hypothesis was not rejected. Table VII includes the data and results relative to hypothesis #6.

TABLE VII

A COMPARISON OF MISCARRIAGE RATE AND THE PRESENCE OR ABSENCE OF CHLOASMA GRAVIDARUM IN POSTPARTUM PATIENTS WHILE HOSPITALIZED

Subjects	N	No. of Patients Who Had Miscarried	No. of Patients Who Had Not Miscarried	Chi Square
With Chloasma	15	6	9	1.62
Without Chloasma	31	4	27	Difference is not significant

Hypothesis #7: There is no significant difference in the degree of postpartal flow for women who have chloasma and those who do not have chloasma.

This hypothesis was rejected. The number of perineal pads that were saturated with blood were recorded on most of the charts soon after delivery. By recording the delivery time and the time the pads were changed it was possible to know approximately how many pads were saturated per hour for the first one or two hours. A rating scale was used to judge the amount of postpartal flow. (See Appendix A)

On four charts this information was not specific enough to use in the study, so that the control group was reduced to twenty-seven for testing this hypothesis.

The chi square (X^2) test of significance indicates that the difference between the two populations was significant at the one per cent level; that is, this

difference between the means could not be expected to occur more than one per cent of the time in the general population of postpartal patients. The null hypothesis was rejected. The data and results for hypothesis #7 are included in Table VIII.

TABLE VIII

A COMPARISON OF POSTPARTAL FLOW AND THE PRESENCE OR ABSENCE OF CHLOASMA GRAVIDARUM IN POSTPARTUM PATIENTS WHILE HOSPITALIZED

Subjects	N	Very Heavy	Heavy	Moderate	Scant	Chi Square
With Chloasma	15	1	1	7	6	15.26
Without Chloasma	27	0	3	15	9	The difference is significant

Hypothesis #8: There is no significant difference in the presence of ketones in the urine for women who have chloasma and those who do not have chloasma.

This hypothesis was rejected. A routine urine specimen was run on all patients who were admitted for delivery. Each specimen was checked for ketones so that this information was on each chart.

The chi square (X^2) test of significance indicates that the difference between the two populations was significant at the 0.1 per cent level; that is, the difference between the means could not be expected to occur more than 0.1 per cent of the time in the general population of postpartal patients. The null

hypothesis was rejected. The data and results are included in Table IX.

TABLE IX

A COMPARISON OF KETONES IN URINE AND THE PRESENCE OR ABSENCE
OF CHLOASMA GRAVIDARUM IN POSTPARTUM PATIENTS
WHILE HOSPITALIZED

Subjects	N	With Ketones	Without Ketones	Chi Square
With Chloasma	15	9	6	22.09
Without Chloasma	31	8	23	The difference is significant

C. Summary and Conclusions of This Study of Patients With Chloasma

The degree of chloasma gravidarum was rated in forty-six postpartal patients and information that was available on patients' charts recorded and classified for differences and similarities. Fifteen patients were classified as having chloasma and the data compared to the data of the thirty-one patients who did not have chloasma.

Analysis of the data revealed that women who have chloasma gravidarum do not show a significant difference from women who do not have chloasma in such factors as: 1) hematocrit, 2) premature birth of infants as measured by the index of infant birth weight, 3) the delivery date compared to the due date, and 4) the miscarriage rate.

Analysis of the data revealed that women who have chloasma gravidarum

do show a significant difference when compared to women who do not have chloasma gravidarum in such factors as: 1) age, 2) gravida, 3) postpartal flow, and 4) the presence of ketones in the urine.

Because of the small size of the sample nothing conclusive can be stated about the incidence of chloasma gravidarum in the general population. All of the findings can be stated only about the patients that were included in this study of postpartal patients. This study opens many possibilities for future research in which many of the variables would be controlled. Further discussion of some of these possibilities is included in Chapter VIII.

PART IV

THE RESULTS

The problem of this study was stated relative to the two main segments: chloasma gravidarum and vitamin E deficiency. Using open-ended research the purposes of this study were: 1) to accumulate and classify data relative to chloasma and vitamin E deficiency, and to show possible relationships (Part II); 2) to generate hypotheses relative to vitamin E deficiency and chloasma gravidarum (Part IV); and 3) to identify and indicate possible implications for nursing (Part IV).

Part III presents an investigation that is the outgrowth of the literature survey. It includes the investigation of eight null hypotheses with data collected from a small sample of postpartal women.

Part IV is an attempt to synthesize the varied findings of the literature survey and the observations of patients with chloasma gravidarum into a related whole. It is an evaluation of the results of the study in the light of the problem and purposes of the study. In it are listed hypotheses and recommendations for further study and statements of implications for nurses and other health personnel. These hypotheses and statements of implications are the outgrowth of this open-ended research design.

CHAPTER VIII

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

A. Summarization of the Segments of This Study

The case of chloasma that seemingly responded to the use of vitamin E along with a balanced diet that aroused the initial interest in this problem (pages 3-4), provided the focus for this open-ended study. However, findings on the segments of the problem do contribute some enlightenment that may lead to knowledge concerning this problem. The parts of this problem include:

1. What factors are known about vitamin E--its functions and its relationship, if any--to pathology?
2. What is the clinical picture of vitamin E deficiency?
3. What is known concerning chloasma gravidarum? What symptoms do patients manifest who exhibit this phenomena?

Do women who have chloasma gravidarum (and their infants) have or develop one or more of the various symptoms or conditions of vitamin E deficiency as it is depicted in the literature? This question cannot be answered without more research.

Vitamin E Deficiency. What factors are known about vitamin E--its functions and its relationship, if any--to pathology? Vitamin E functions intracellularly in a ratio relationship to unsaturated fatty acids. Variation in the need for tocopherol is dependent upon other variable factors such as enzyme control that may be affected by genetic errors, the level of unsaturated fat in the diet, the duration of the depletion of tocopherol, and stressor situations that increase the need for vitamin E. Prenatal diet in animals can affect the polyunsaturated fat stores in the newborn infant, or in newly hatched chicks making them, more

or less, susceptible to vitamin E deficiency. Since the encephalomalacia, that is often present in vitamin E deficient chicks, has also been seen in at least one human infant, the species specificity that is characteristic of tocopherol deficiency, should not be used to eliminate the possibility of varied pathology in humans. Vitamin E deficiency has been clinically identified in humans in many forms. Because it functions intracellularly in all types of cells, it can affect all types of tissues and systems. Focal necrosis sometimes occurs. Which system that might be effected and the amount of vitamin E needed to prevent deficiency may be determined genetically. The variability of the many factors related to vitamin E deficiency make it difficult to set standards for the requirements of vitamin intake at levels that prevent deficiency.

The functions of vitamin E are listed on page 60. The emerging clinical picture is described on pages 60-61. The one predominating characteristic common to the varied forms in which vitamin E deficiency manifests itself is steatorrhea--excessive fat in the feces. Vitamin E deficiency seems to be interlinked with faulty fat metabolism. For almost thirty years researchers have been noting a pigmentation in the tissues of vitamin E deficient animals that seems to be the result of increased oxidation of the cellular lipoproteins. Increased skin pigmentation occurs with aging and is known as lipofuscin--similar to the ceroid deposit that is often found in E deficient tissues. Much is now known about vitamin E deficiency but there is still much unknown--especially at the biochemical level. Vitamin E deficiency is related to human pathology in many ways.

Chloasma Gravidarum and Pigmentation. This investigator was unable to find evidence, that chloasma gravidarum is associated with vitamin E

deficiency in the accepted medical literature. A discussion of melanin, its function and how it is influenced is included in the first part of Chapter IV. Melanin is influenced by MSH (melanin stimulating hormone). MSH influences skin pigmentation and may influence fat metabolism and evokes responses in nerve cells. Melanin promotes the nonenzymic oxidation of the intermediates of a cell process. This points to the need for additional antioxidant during pregnancy.

The Study of Postpartal Women. The study of postpartal women (Chapter VII), that was the outgrowth of open-ended research revealed that there is a significant difference in women who have chloasma gravidarum and those who do not, in several ways. Among the women in this sample, those who had chloasma: are older, are more likely to be multiparas, have ketones in their urine and have a heavier immediate postpartal flow the first two hours after delivery, than do women who do not have chloasma. While their infants were not premature, the data revealed that infants born to women with chloasma are born earlier than those born to women without chloasma at the ten per cent level of significance.

B. Discussion and Recommendations Regarding Study of Postpartum Patients

Age. Since pigmentation is increased in relation to age, it is not surprising that this variable showed a significant relationship to the increased pigmentation of chloasma gravidarum. Future studies could control this variable by using a much larger sample and study only those in the younger age group.

Gravida (Pregnancy). It is likely that this variable is interrelated to the age factor. This variable could also be clarified in the same manner as

suggested for controlling for age. Also, the number of children could be included as having stressor weight in a scale for measuring stress in the child-bearing year. A problem that could be researched is: do women (aged 18-25) who are multiparas have chloasma gravidarum more frequently than women who are not multiparas?

Hematocrit. Because hemolytic anemia has been seen in patients who are vitamin E deficient, it was desirable to have some blood index. However, the hematocrit was the only blood index that was consistently on all the charts. Other blood indices, such as the reticulocyte count, hemoglobin and erythrocyte stability (since these were affected in Oski's vitamin E deficient infants), might be of more value.

Prematurity with Infant Weight as an Index. Prematurity is defined by the World Health Organization by specifying specific criteria. When using birth weight as a criteria for prematurity, anything less than 2,500 grams (five pounds and eight ounces) or less is considered to be premature.¹ The mean average weight of the infants born to these mothers with chloasma gravidarum is 6.59 pounds as compared to 6.77 pounds for those without chloasma.

Future studies could measure other criteria more specifically using other specified indices such as height, head and chest measurement, etc., and come to a clearer definition of prematurity and then compare this to the presence and absence of chloasma in the mother. Dr. Oski's low weight infants were vitamin E deficient. Were their mothers prenatally also vitamin E

¹Dorothy M. Stewart, and Pauline Vincent, editors, Public Health Nursing: A Book of Readings (Dubuque, Iowa: Wm. C. Brown Company, 196), pp. 213-217.

deficient?

Prematurity as Measured by the Due Date. If this measure were used with a larger sample with other variables controlled, its borderline significance might be clarified. The due dates are usually on charts and birth dates (if not influenced by induction of labor) might be a valuable way to establish the amount of prematurity.

Miscarriage. Since Oski's low weight infants were considered premature and were vitamin E deficient; and if chloasma gravidarum is related to vitamin E deficiency, this investigator hypothesized that women who have chloasma would miscarry more frequently than those who do not have chloasma gravidarum. In analyzing the collected data of postpartum patients and testing for significance of the difference in their miscarriage rates by chi square, the difference between the means did not support the idea.

Postpartal Flow. If women who have chloasma are vitamin E deficient, they might also exhibit hemolytic anemia that sometimes accompanies vitamin E deficiency. This might be reflected in the degree of postpartal flow. Not enough is known to make this assumption. Further research may show relationship. All that can be said at this point is that in these two samples the women who had chloasma had a heavier postpartal flow than those without chloasma.

Ketones. When the incidence of ketones was discovered in larger numbers among the "with chloasma" group, a cause was sought. None of these patients with or without chloasma had diabetes recorded as a diagnosis on their charts. This ruled out at least one variable. The urine specimens were collected on admission or on the delivery table. It has been assumed that the ketones present in the urine of many pregnant women might be related to low

food intake the day of labor and thus the patient would burn up body stores of fat resulting in ketones in the urine. This does not explain the significant difference found when these two groups of patients were compared. Why did one group show such a significant level of ketones? (Table IX, p. 82). Both groups had an equal reason to not eat food, so this cannot explain the difference.

Ketones are produced in relation to increased fat metabolism is about all that can be stated at this time.

C. Repetitious Factors.

Starting with the separate segments of this study they all lead eventually to one common factor--fat metabolism. 1) In vitamin E deficiency the one common characteristic is steatorrhea. The need for the fat soluble vitamin E is closely linked to unsaturated fatty acids. 2) MSH (melanin stimulating hormone) in addition to influencing skin pigmentation, may have some function in regulating fat metabolism; and it is increased in the urine of pregnant women. 3) Ketones, an end product of increased fat metabolism, are often in the urine of pregnant women. There is a significant difference when comparing these postpartum patients with chloasma and without chloasma. Could chloasma gravidarum be a symptom of faulty fat metabolism? Could it possibly signify an unbalanced vitamin E/polyunsaturated fatty acid ratio? These questions are for the biochemist. However, the observations had to be made in the clinical setting in relation to an observed phenomenon. Nurses can become members of research teams and thus bring together many points of view and this might increase the breadth of perspective.

Another repetitious set of interrelationships is apparently related to

pregnancy, pigmentation, schizophrenia, and Addison's disease. 1) Along with increased pigmentation, there is increased MSH (melanin stimulating hormone) in the urine of pregnant women and persons with Addison's disease. 2) Schizophrenics have a predominance of dark hair and they have a breakdown product of adrenalin in their systems. 3) Addison's disease is related to the hypofunction of the adrenal cortex. 4) Melanin and adrenalin come from the same chemical precursor. 5) Schizoid-like behavior is seen in pregnant women, and Addison's disease as well as in schizophrenics. While this investigator does not even pretend to understand these interrelationships, somehow, they seem to be connected. If one were to add serotonin to this list of variables, it might lead in a specific direction. It is seen in increased amounts in schizophrenics, in pregnant women and decreased in phenylketonuria--that can produce mental retardation. See (Table I, page 74) the diagram of the directions that tyrosine can go. In phenylketonuria, there is an inborn error that affects the metabolism of phenylalanine.

Discussion. The Findings of this paper have revealed more questions than answers; but the questions must be asked if there are to be any answers. Research could be done at the 1) biochemical level, 2) at the behavioral level, and 3) at the clinical nursing level. What is the chemistry of melanin production and of vitamin E deficiency? Much has been done but there are still many unanswered questions. How does the biochemistry of vitamin E deficiency and melanin production affect the behavior of persons? Caplan made some observations about pregnant women and their behavior. Was there a difference in the amount of pigmentation or chloasma gravidarum in the women he observed? Would women who have chloasma gravidarum show a significant increase of

schizoid-like behavior? What phenomena have no apparent causes, and yet persons affected by them have multiple problems? Could these phenomena be broken down into segments for study but care used in bringing the segments back together again for evaluation?

The knowledge explosion that has resulted from researching segments is valuable but can be valueless if it remains in isolated fragments. Some researchers need to become involved in pulling together the fragments of research into a harmonious whole. This paper has, through the use of open-ended research, attempted to bring the multiple pieces into a related whole in order to show relationships. It has relied heavily on the researched segments of others.

The "mask of pregnancy" or chloasma gravidarum appears to be significant as a possible symptom of disease or deficiency. The degree of its significance and what it is a symptom of is still undetermined. That it might have a relationship to vitamin E deficiency and fat metabolism is revealed in this paper. Laboratory evaluation of tocopherol serum levels in women who have chloasma gravidarum is necessary before the relationship of these two factors can be established. Longitudinal studies need to be done to determine if persons who have chloasma do have or do not have more illness than those who do not have chloasma. These studies could continue into the next generation and the children and grandchildren of persons who have chloasma, as well as a control group, to see what similarities and differences occur.

D. Hypotheses For Future Study

1. Women who have chloasma gravidarum have a lower tocopherol serum level than women who do not have chloasma gravidarum.
2. Women who are vitamin E deficient give birth to smaller infants than do women who are not vitamin E deficient.
3. Women who are vitamin E deficient have more unsuccessful pregnancies than do women who are not vitamin E deficient.
4. Women who have chloasma gravidarum have a higher incidence of steatorrhea than women who do not have chloasma gravidarum.
5. Women who have chloasma gravidarum give birth to infants that are more hypersensitive than are the infants born to women without chloasma gravidarum.
6. Women who have chloasma gravidarum give birth to infants that develop eczema or skin rashes more frequently than do women who do not have chloasma gravidarum.
7. Women who have chloasma gravidarum are more inclined to fantasize and to develop schizoid-like behavior patterns than do women who do not have chloasma gravidarum.
8. Women who have chloasma gravidarum are more likely to develop symptoms that are related to nervous system degeneration than women who do not have chloasma gravidarum.
9. Women who have chloasma gravidarum are more likely to develop "postpartal blues" than women who do not have chloasma gravidarum.
10. Women who have chloasma gravidarum are more likely to develop hemolytic anemia than women who do not have chloasma gravidarum.
11. The children born to women who have chloasma gravidarum are more likely to have a blotchy pigmentation of the skin over the malar region of the cheek until after adolescence than do children born to women who do not have chloasma gravidarum.
12. Diseases of adaptation are more likely to occur in families in which the mother had chloasma gravidarum than in families in which the mother did not have chloasma gravidarum.

13. Women who space their infants at least three years apart are less likely to develop chloasma gravidarum than do women who give birth to infants in rapid succession.
14. Women who have chloasma gravidarum are more likely to have bizarre appetites than do women who do not have chloasma gravidarum.
15. Women who have chloasma gravidarum are more likely to have significant hair loss than do women who do not have chloasma gravidarum.
16. Retinal neuropathy occurs more frequently in women (and their children) who have chloasma gravidarum than in women who do not have chloasma gravidarum.
17. Addison's disease occurs more frequently in women who have had chloasma gravidarum than in women who have not had chloasma gravidarum.
18. Women who have chloasma gravidarum follow diets that include more polyunsaturated fat than do women who do not have chloasma gravidarum.
19. Women who have chloasma gravidarum are more prone to give birth to infants who develop hemolytic anemia.
20. In families in which the history includes both diabetes and heart disease, chloasma gravidarum is more prevalent than in families in which these illnesses are not included as a part of the family history.
21. Women who have chloasma gravidarum are more likely to have an irregular mensis than women who do not have chloasma gravidarum.
22. Women who have chloasma gravidarum have an earlier menopause than do women who do not have chloasma gravidarum.
23. Mental illness is more prevalent in women who have chloasma than in those who do not manifest chloasma gravidarum.
24. The incidence of generalized illness is more prevalent among women who have chloasma gravidarum than in women who do not have chloasma gravidarum.
25. Chloasma gravidarum is more likely to occur in family groups than in the general population.

26. The degenerative diseases are more prevalent in the histories of persons who have chloasma gravidarum than in those who do not have chloasma gravidarum.
27. Infants born to women who have chloasma gravidarum are more likely to develop hepatic dysfunction than those born to women who do not have chloasma gravidarum.
28. Women who have chloasma gravidarum have a more rapid pulse rate than women who do not have chloasma gravidarum.
29. Women who have chloasma gravidarum are more likely to have dark hair than women who do not have chloasma gravidarum.
30. Infants born to women who have chloasma gravidarum sleep less than infants born to women who do not have chloasma gravidarum.
31. Women who have chloasma gravidarum have a mean temperature level that is higher than the mean temperature level of women who do not have chloasma gravidarum.
32. Women who have chloasma gravidarum have trouble with varicosities more frequently than women who do not have chloasma gravidarum.
33. Women who have chloasma gravidarum have a higher blood pressure mean than do women who do not have chloasma gravidarum.
34. Women who have chloasma gravidarum require a longer period of time to reach their former level of wellness following the birth of a child than do women who do not have chloasma gravidarum.
35. Women who have chloasma gravidarum are more frequently hospitalized for treatment of illness during the first year following the birth of a child than are women who do not have chloasma gravidarum.
36. Chloasma gravidarum has a higher incidence in geographic areas in which the selenium content of the soil is low than in areas where the selenium content of the soil is high.
37. Crises occur more frequently at the developmental transition periods in children born to women who have chloasma gravidarum than in women who do not have chloasma gravidarum.

38. Infants born to women who have chloasma gravidarum develop pitting edema more frequently than infants born to women who do not have chloasma gravidarum.
39. Heart disease is more prevalent among women who had chloasma gravidarum than in women who did not have chloasma gravidarum.
40. Asthma occurs more frequently among the children born to women who had chloasma gravidarum than in women who did not have chloasma gravidarum. (Epithelial tissues involved.)
41. Women who have chloasma gravidarum give birth to infants who later develop multiple sclerosis more frequently than do women who do not have chloasma gravidarum.
42. Multiple sclerosis occurs more frequently among women who have chloasma gravidarum than in women who do not have chloasma gravidarum.

This does not exhaust the possibilities for research. Perhaps the reader could continue adding to this list. If chloasma were proved to be related to vitamin E deficiency, then all the conditions related to E deficiency would be related to the absence or presence of chloasma gravidarum.

E. Tools To Be Developed and Validated

A tool that would be of value in doing research relative to vitamin E deficiency would be a rating scale by which to evaluate the stressors of the child bearing year. For example, perhaps twins could be considered a double stressor in a scale that evaluated the stressors of the child bearing year.

The rating scale (Appendix A) that was developed to assign the degrees of chloasma present needs to be validated with a larger sample and perhaps improved. It could be used to evaluate patients for chloasma so that this symptom could be recorded for diagnostic and research purposes.

F. Implications for Nurses and Other Health Personnel

The foremost recommendation for nurses is that chloasma gravidarum be recorded on the charts so that research can be done relative to chloasma gravidarum. This can be done without arousing undue concern, since the patient need not know about it unless the doctor wants the patient to know it.

Since the science of nutrition involves the nature and amount of the dietary constituents required for the synthesis and replenishment of enzymes and their prosthetic groups; and since the science of physiology is focused on processes such as active transport, muscular contraction, and nerve conduction; and since these processes are enzyme-mediated; good nutrition is essential to good health. Professional persons need to understand nutrition at the biochemical level. High level health may become thought of at the "molecular level" rather than the "cellular level."

It is of value to nurses to know that, though current information lists the dosage for vitamin E as unknown, recommended daily dosages have ranged from five to thirty milligrams. Therapeutic doses range from fifty mg. and above.

In counseling concerning nutrition it is necessary for the nurse to consider such things as inborn errors of metabolism, the availability in food stuff of certain molecules, the actual intake of various food components, and the ability to utilize available vitamins. Does steatorrhea drain the fat soluble vitamins from the system before they can be utilized? Vitamin E is fat soluble. The ratio between polyunsaturated fats and vitamin E are important in considering ways to prevent tocopherol deficiency.

Keeping up with nutrition research is important if adequate counseling

of the patient is to take place. The nurse needs to know about the adequate sources of vitamin E (page 65); how processing affects the level of content (pages 65-66); and how much and which is considered to be an adequate intake. This information is not presently available but researchers are trying to determine requirements that are adequate to prevent deficiency. In the meantime, a balanced diet that includes foods that contain vitamin E is important. While the nurse cannot be a specialist in all areas, she needs to seek the cooperation of the nutritionist or dietitian in planning health care.

Since food faddists have practically adopted vitamin E as their project, it is more important than ever for nurses to be aware of the facts, so that they can help patients determine fact from fancy. Vitamin E is not a cure-all.

One discovery of this study is that the need for vitamin E and the consequences of its deficiency seem to be ignored by medical doctors in the United States. They seem to think of it as "the antisterility vitamin," Perhaps this is because of the conflicting reports in research and the complexity of determining the level of dosage needed. "A Problem that cannot be solved had better be ignored," seems to be a prevalent attitude. Much of the research is being done in other countries; though, Horwitt and others have made some valuable studies at Evanston, Illinois and Oski's study on Vitamin E deficient infants was done at Pittsburgh, Pennsylvania. Perhaps published reports of valid research that shows the multiple relationships would aid in bringing this research problem to their attention.

Processed foods that have been enriched with vitamins have not included vitamin E. Because of this, there may be a vitamin E deficiency in the general population who depend on processed foods.

The awareness of nurses of the symptoms of vitamin E deficiency is necessary if accurate reporting is to occur. Relative to vitamin E deficiency, sound diagnosis needs to be made and treatment needs to be based on sound judgment.

So far, diagnostic procedures necessary for identifying vitamin E deficiency are done only in research laboratories. It would be of value for nurses to be aware of the possibility of laboratory diagnosis (page 62). Since clinical diagnosis is not always in agreement with laboratory diagnosis (this may be based on individual differences in need and utilization), the observation and reporting of clinical symptoms is more important (page 62).

It is important for the nurse to know that synthetic tocopherol has difficulty in crossing the placental barrier as compared to the natural product. This is especially important since vitamin E deficiency can affect the development of the fetus.

It is important for the nurse to know that processing of infant foods can destroy the vitamin E or cause it to deteriorate over a period of time (pages 65-66). Rancidity destroys vitamin E.

Nurses need to recognize the need for the prenatal patient to talk about repressed material or fantasies, that Caplan discusses as a phenomenon and that occurs during pregnancy and lasts for several weeks following pregnancy; they need to accept the fact that the schizoid-like behavior is a prenatal pattern that will usually pass following pregnancy and that it is probably related to the chemical changes of the child-bearing years. If, however, it does not pass, it needs to be reported to the doctor so that the patient can receive help.

Patients with chloasma gravidarum may be embarrassed about "their mask." Listening to them may be all they need. For many persons it disappears immediately following pregnancy. One person in the sample observed, had only a faint mask. On her fourth postpartum day it was no longer identifiable.

Extended knowledge about vitamin E deficiency will be of value to nurses in aiding them: 1) to identify signs and symptoms for reporting and recording; 2) to recommend a diet that includes a balance of all known vitamins; and 3) to gain an understanding of the possible limiting factors in the utilization of vitamin E (VI).

G. Conclusion

The basic problem of this study could not be researched. The question still stands: Do women who have chloasma gravidarum (and their infants) have or develop one or more of the various symptoms or conditions of vitamin E deficiency as it is depicted in literature? Additional funds and skills, that were not available to this investigator, are necessary before this question can be answered. If, however, enough attention has been drawn to this problem to stimulate research, and to the recording of chloasma gravidarum as a symptom is begun, then this study has served a purpose. The emerging clinical picture as depicted in literature will aid in identifying the varied symptoms of vitamin E deficiency.

Each segment of this study led eventually to fat metabolism: 1) vitamin E is fat soluble and its deficiency is associated with steatorrhea; 2) melanin stimulating hormone, that regulates melanin production, may also be involved in regulating fat metabolism and in influencing neural mechanisms; and

3) ketones in the urine are related to increased fat metabolism.

An exploration of the little known about chloasma gravidarum led to the study of forty-six postpartum patients in a hospital setting. Of these patients, fifteen had some degree of chloasma and thirty-one did not. In addition, it revealed that women who have chloasma are inclined: 1) to be older; 2) to be multiparas; 3) to have a heavier postpartal flow; 4) to have ketones in their urine; and 5) though their infants are born a little earlier, it is not at a significant level.

If chloasma gravidarum is a symptom of significance, as the ketones in the urine of the postpartum women in this study indicate, it may have value in alerting health personnel to the disease or deficiency of which it is a symptom, before it has become a serious health problem.

-102-

APPENDIX A

INFORMATION COLLECTION FORM

NAME (indicated by #) _____ Dr. _____ Age _____ Observed
PP Day _____

MASK: faint _____ moderate _____ marked _____ absent _____

Pregnancies: 1, 2, 3, 4, 5, 6, 7, 8.

This Pregnancy:

Ht. Wt. _____

Father _____

Mother _____

Infant _____

Infant _____

PP Flow:

Menarch _____ every _____ days for

_____ days. Cramps? _____

LMP _____ Quickening _____

EDC _____ Birth Date _____

Pregnancy Duration _____ wks.

Gravida _____ Para _____ Miscarriages _____

Family History:

Personal History:

Present State of Health:

Blood:

Rh _____

Type _____

Hgb. _____

Hct. _____

Other: _____

Urine:

Sp. Gr. _____

Reaction _____

Albumin _____

Sugar _____

Ketones _____

Microscopic:

Mucous _____

Epithelial _____

WBC _____

RBC _____

RATING SCALES

Scale for rating the degree of chloasma gravidarum present in postpartum patients.

The degree of discoloration was rated as follows:

1. Absent. Chloasma not identifiable.
2. Faint. A light discoloration across the cheek prominences.
3. Moderate. Brown discoloration extends to the forehead or down over the cheeks and malar region.
4. Marked. Discoloration extends to both the forehead and malar region.
5. Very marked. The color is more pronounced and may be present around the mouth or extend to other parts of the body.

Scale for rating the amount of postpartal flow for first two hours.

1. Very Heavy. More than four pads saturated per hour.
2. Heavy. From two to four pads per hour.
3. Moderate. From one to two pads per hour.
4. Scant. Less than one pad per hour.

The method of determining the amount of postpartal flow was to determine the difference between the time of the infant's birth and the time when the pads were changed for the first time. The number of pads were recorded on the chart so that it was possible to determine the number of pads saturated per hour and to rate their flow according to the above scale.

APPENDIX B

In order to understand the possible interrelationships of chloasma gravidarum--the blotchy yellow-brown pigmentation of the skin that sometimes occurs during pregnancy--and the multi-faceted dimensions of vitamin E deficiency, as it relates to the pregnant woman and her infant, it is necessary to understand, at least in part, the body cells and their many processes. It is also necessary to review the interrelationship of enzymes, hormones and vitamins because of their dependency upon each other.

This appendix is provided to aid the reader with a quick review if it would be helpful.

THE NORMAL CELL

A. The Cell--A Structural Unit

Since vitamin E functions intracellularly, this section will summarize the normal cell and its processes. The cell is a structural unit of life in which the processes of life occur. When it is "damaged, and that damage cannot be repaired, it is but a matter of time before all activity will come to a halt and disintegration of the working parts will ensue."¹ Cells vary widely in size, shape, and function; but all cells have essentials without which no cell can survive. The three systems around which the biochemistry of the cell center are (1) the membrane system, (2) a system for reproduction, and (3) a system for powering the activities.²

The Mechanisms of the Cell. Each body cell has its own independent machinery within the cytoplasm of the cell. That is, it has (1) its own power plant--the mitochondria produces the energy necessary for that specific cell by converting sugar into energy; (2) a factory district--the ribosomes manufacture the protein compounds which form a major part of every organism including the enzymes, which are protein structures; (3) a packaging and distributing complex--the golgi complex deals with the proteins that are manufactured and turns them into units that can be distributed outside the cell; (4) its own disposal unit--the

¹David E. Green, and Robert F. Goldberger, Molecular Insights Into The Living Process (New York, London: Academic Press, 1967), pp. 2-3.

²Ibid., pp. 3-4.

lysosomes that digest nutrients and dissolve cellular waste; (5) a transport system--the endoplasmic reticulum (ER), which is suspended and swaying in the cytoplasm. It is a network of segmented micro tunnels on which some of the ribosomes are attached; and (6) within the nucleus of the cell is a jelly-like mass, chromatin. Here, the engineer is deoxy-ribonucleic acid (DNA)--a spiral stair-way with steps that are a combination of the molecules known as neucleotides, adenine, guanine, cytosine and thymine. The structure and type of the individual and all his parts--to the smallest part of each of his cells--are in this genetic blueprint; (7) the messenger--ribonucleic acid (RNA) which unzips from DNA, transfers the pattern and travels out of the nucleus, utilizing amino acids to make up proteins.

This description of the cell's machinery, or organelles made up largely of lipoproteins, is simplified to bring the whole complex into focus.³

B. Processes That Are Universal to Cells

Within the cell, the systems that generate and utilize energy are variations of a single system. This system involves a cyclic interaction of molecules in which the progressive steps use the end products of the preceding step as the material for the next. Each step produces energy--in the form of ATP (adenosine-5-triphosphate)--for the functioning of individual cells. In the animal kingdom, the mitochondrion and the glycolytic cycle are the coupling mechanisms for the

³This analogy is a combination borrowed from John Pfeiffer, Life Science Library, "The Cell" (New York: Time Incorporated, 1964); and from A. L. Tappel, "Where Old Age Begins," Nutrition Today, 2:4, December, 1967.

synthesis of ATP.⁴

Other Biochemical Universals. Other biochemical universals include (1) metabolic systems--these process available matter into usable units; (2) membranes--the vesicular or tubular lipoprotein systems of fused repeating units with a structured periphery and a fluid interior form the organelles to carry out the functions of the cell; (3) protein synthesis and cell division as seen in the "chain of command" from DNA to RNA to protein; (the genetic variation of these proteins may influence the ability of individuals to absorb and/or utilize vitamin E); (4) control mechanisms--end product inhibition, repression, and induction; (vitamin E may have a function here) (5) catalytic proteins and the mechanisms of catalysis appear to involve the nature of the active site, the nature of the functional groups which implement the catalysis and the sequence of the catalytic events.⁵ These mechanisms of catalysis must fit like a key in a lock if the processes are to be completed and if the cell is to function normally.

The Aging of the Cell--A Normal Process. Aging is a normal process of the cell. There is a "tug of war" between the forces that age the cell and the protective mechanisms that prevent the aging of the cell. Vitamin E functions at the intracellular level to protect the polyunsaturated lipids of which much of the cell is composed. It acts as an antioxidant and prevents premature aging. A. L. Tappel states that peroxidation of the polyunsaturated lipids involves the direct reaction of oxygen and lipid to form free radical intermediates. "Lipid

⁴Green, op. cit., pp. 385-389.

⁵Ibid., pp. 386-388.

peroxidation is, therefore, widely regarded as the main spring in the aging process."⁶

In the above cited reference Dr. Tappel included pictures depicting the cell at various ages to show the increased pigmentation with increased age.⁷

Cellular aging as pictured by Dr. Tappel can be summarized in four stages, as follows:

1. Radiant energy penetrating cell membrane initiates lipid peroxidation, releasing free radicals.
2. A free radical collides with a lysosome (see page 39) puncturing its membrane and allowing the release of hydrolytic enzymes.
3. The hydrolysate spreads through the cell, destroying cellular components. Another free radical ruptures the cell membranes.
4. The end product of lipid peroxidation: a ruptured cell membrane, a distorted nucleus, and of the lysosome, only a "clinker" remains. The host has become one cell older.⁸

Slowing down the aging process may be a possibility by the adequate use of antioxidants of which vitamin E is one. Vitamin E is a free radical scavenger can avert much harm to individual cells and, thus, the body.

⁶A. L. Tappel. Recent research has revealed many facts that help scientists piece together fragments of older researched phenomenon and to come up with new theories. Dr. A. L. Tappel, Professor of Nutrition and Professor of Food Science and Technology, at the University of California at Davis, has developed a theory relative to the aging of the cell.

⁷Thirty-five millimeter slides of the illustrations in Tappel's article are available upon request from: Director, Education Services, Nutrition Today, 1140 Conn. Ave., N.W., Washington, D.C. 20036.

⁸Tappel, op. cit., p. 4.

C. A Framework for Understanding High Level Wellness

The universals of the cell give a framework for understanding life and various levels of wellness. The health of the cell is affected by the sum total of its parts and processes--the atoms, molecules, macromolecules that make up its systems, and the catalysts and prosthetic groups that promote essential processes.

The availability of the essential nutrients, of which vitamin E is one, and the correct assembly of the atoms of elements that make up the cell (with its machinery) are essential for a high level of wellness. Absence of essential elements would cause the death or maladaptation of the cell and eventually the tissue, organ, or individual of which that cell is a part.

Nutrition as a Source of Elements.

The science of nutrition involves the nature and amounts of the dietary constituents required for the synthesis and replenishment of enzymes and their prosthetic groups....Among the key materials whose synthesis is controlled by the hereditary apparatus are the enzymes....Much of the science of physiology is focused on processes such as active transport, muscular contraction, and nerve conduction--processes which are uniformly enzyme mediated.⁹

Unless the elements are available for the synthesis of enzymes, physiological processes that are enzyme mediated cannot function normally. Some research has been done to determine if possibly vitamin E is needed for enzyme production, and in this way affect cell processes.

While it is supposed that most of the essential nutrients have probably been identified, very little is known about how many of these nutrients perform their functions and the relation of these functions to health, aging, and

⁹Green and Goldberger, op. cit., p. 88.

resistance to disease. Some misconceptions about malnutrition and its causes persist in spite of much new knowledge. One current nutrition text indicates that because vitamin E is prevalent in food, that those who eat an otherwise adequate diet, probably get enough vitamin E. However, Sebrell disagrees.

The science of nutrition is no longer a study of the deficiency diseases of poor and ignorant people, produced by lack of food. Most of the clinical malnutrition (in the U.S. today) is "conditioned malnutrition" secondary to physical, mental, or physiological stress of many kinds, often occurring as a complication of other diseases, in which economic conditions and the availability of food are of minor importance.... (while reports) supported by the National Vitamin Foundation, have shown that many people are nutritionally below average by dietary, biochemical, and clinical criteria. One cannot make the sweeping generalization that all Americans have so much food available that they consume all the nutrients they need.... The changing concept of malnutrition is placing it in the category of the metabolic abnormalities which have been produced as the result of some other disease. He (the physician) must also be able to recognize the typical deficiency diseases as they will continue to occur... (and) be on the lookout for their ever increasing metabolic abnormalities of genetic origin.¹⁰

D. The Interrelationship of Hormones, Enzymes and Vitamins

Vitamins, enzymes and hormones are closely related in terms of their necessary interaction.

Vitamins are nutrients or chemicals contained in food essential for the formations of enzymes and their prosthetic groups, and they sometimes act as coenzymes. Green and Goldberger state that

vitamins are small organic molecules which are required for the normal metabolic activities of a given organism, but cannot be synthesized by that organism. In the event that they are inadequately supplied from an external source, disease will result.... Often, especially if the disease is discovered early in its course, a cure can be effected by the administration of the appropriate vitamin. Basic research on the structure and function of the vitamins has contributed greatly to our understanding of the biochemical

¹⁰W. H. Sebrell, Jr., "Changing Concept of Malnutrition," The American Journal of Clinical Nutrition, 20:6:653-658, June, 1967.

basis of the vitamin deficiency diseases. Many of the vitamins have been found to function as coenzymes or as prosthetic groups, or to be essential to the synthesis of such groups in vivo. These groups are parts of enzymes involved in important metabolic activities.¹¹

Enzymes are proteins that act as triggers to initiate cell processes; they create a right climate for the reactions essential to cell functioning. They are not a part of the chemical change that occurs, but much of the time the processes would be too slow without the aid of a catalyst--an enzyme--to speed up the process. The synthesis of enzymes is controlled by the hereditary mechanism of the cell. For each step of the cell's processes there is an enzyme.¹² Green and Goldberger state that "the great majority of enzyme-catalyzed reactions are reversible; (this contributes to homeostasis in the cell). No one cell contains all of the known enzymes." They have classified enzymes into five categories according to the reaction they catalyze.¹³ The number of enzymes is still not known, but there are an estimated 100,000 in the human body. More than 700 have been isolated and much research is being done to understand their specific functions.¹⁴

¹¹Green and Goldberger, op. cit., pp. 344-345. Coenzymes are minute factors that must be linked to some enzyme for the enzyme to function. Many coenzymes are vitamins or contain vitamins. The organism would die if the co-enzyme were not available since most of the coenzymes play a vital role in the economy of the cell (p. 72). The need for continued intake of vitamins is due to the fact that enzymes have a limited lifetime. (p. 92)

¹²Donald G. Cooley, Modern Medicines (New York: Pocket Books, 1963), pp. 32, 110.

¹³Green and Goldberger, loc. cit. Classification of enzymes: (1) hydrolysis, (2) group transfer, (3) oxidation and reduction, (4) isomerization, (5) condensation.

¹⁴Alton L. Blakeslee, "News," Today's Health, October, 1963. p.

Hormones are biological chemicals produced by the endocrine system in response to a biological need, and are a "variation on a theme" of the chemically triggered control system of the body.

The pituitary gland--the master gland--is about the size of a pea and is located in the center of the head next to the hypothalamus. "There are few functions of the body not influenced at least indirectly by the pituitary."¹⁵ It is composed of three lobes and is responsible for the secretion or control of at least nine hormones (the melanin stimulating hormone-MSH-is one). MSH comes from the intermediate lobe and influences pigmentation. Very little is known about the intermediate lobe. There is a "feedback" regulatory interaction between the pituitary hormones and those of other endocrine glands. "The feedback mechanism is largely mediated through the hypothalamus which is further integrated with the other visceral control centers and even the higher brain centers. Thus, even an emotion may set about a train of events leading to an alteration of some remote gland or organ."¹⁶ Pituitary hormones have some overlapping functions and target organ hormones either supplement or negate each other. The pituitary hormones, as well as other hormones, are specialized macromolecules for specialized purposes, but their influence is far reaching.

Cooley states that "...Vitamins are so many faceted, so interdependent, so interlinked with hormones, enzymes and other molecules, that their effects

¹⁵Joseph R. Dipalma, "The Pituitary Hormones: Potential Wonder Workers," RN, January, 1966, pp. 66-67.

¹⁶Cooley, op. cit., p. 147. Recent research suggests the possibility that the hypothalamus secretes the hormones and the pituitary stores and releases them. In transplantation the pituitary body cannot survive except in close proximity to the hypothalamus.

are manifested in every function of the body. "¹⁷

E. The Influence of Stress on the Aging Process

Within the span of life, stress and adaptation affect cells and their processes. This effect is manifested in many ways, one of which is to deposit pigments in various cells and tissues. Since pregnancy is considered a stressor period for many women, the influence of stressors need to be considered.

The General Adaptation Syndrome. Hans Selye demonstrated that stress has physiological consequences. He described what he called the general adaptation syndrome --G.A.S. He used a variety of "stressors" including chemical and environmental factors, such as prolonged cold and restraint. He showed how rats that were exposed to small doses of a stressor adapted to it. In the next phase of experimentation the stressor was removed for a period of time. Later, when the rats were exposed to the same stressor, they were able to tolerate it better than rats who had not had previous exposure to that stressor.

Selye defined three stages that resulted from the introduction of a "stressor" or "noxious agent." (1) The alarm stage when the body is reinforced chemically to meet the emergency and either succumbs by death or is able to adapt to the stress factor; (2) the adaptation stage or stage of resistance in which the reactions are the opposite of the alarm stage and the body often returns to normal and future encounters with that noxious agent can be met successfully; (3) the stage of exhaustion is reached after prolonged exposure to the noxious agent that produces stress and the body's resources are used up, resulting in

¹⁷Donald G. Cooley, "What is a Vitamin," Today's Health, 41:73, June, 1963.

the death of the individual.¹⁸

Selye states in the preface to his book that "the same stress which makes one person sick can be an invigorating experience for another... (and that) life is largely a process of adaptation to the circumstances in which we exist.... The secret of health and happiness lies in successful adjustment to the ever changing condition on this globe."

Studies on Aging by Selye. Bailey sites a published paper in which Selye reported some experiments relative to stress, aging, and treatment with vitamin E. He produced evidence of aging in rats and compared it to a control group of rats. Then he produced aging in two groups of rats and treated one group with large doses (1,000 mg.) of vitamin E. The treated rats were able to withstand the aging process while the untreated rats died from the noxious treatment.¹⁹

Since pregnancy is considered a stressor period in the life cycle of a woman, does she need more vitamin E to withstand the stressor effects during pregnancy? Is chloasma gravidarum a symptom of the peroxidation that occurs when vitamin E is not adequate to prevent the oxidation of lipoproteins of which the cells are constructed?

¹⁸Hans Selye, The Stress of Life (New York: McGraw-Hill Book Company, 1956), pp. 31, 32.

¹⁹M. Cantin, Jean-Marie Diendonne, and H. Selye, "Effects of Vitamin E on Cardiomuscular Calciphilactic Lesions," Exp. Med. and Surg. (Canada: 1962), 318:20, cited by Herbert Bailey, Vitamin E: Your Key to a Healthy Heart (New York: ARC Books, Inc., 1964), pp. 94-96.

F. Summary of Appendix B

Metabolic processes occur in the cell, the basic unit of life. Enzymes trigger these processes. Specialized cells secrete specialized macromolecules-- hormones--to perform specialized tasks. The cell can function only if it is provided with essential elements and nutrients. Vitamins are chemicals essential for the production of enzymes.

The interdependence of vitamins, enzymes and hormones as a part of the chemical mechanisms for integrating myriads of cell activities for the welfare of the body, are becoming better understood. Research relative to the aging process is yielding some of its secrets. The aging of the human organism (a normal process) appears to be influenced by an "intracellular tug of war."

New concepts of malnutrition are at the biochemical level and the naive concept that because there is adequate amounts of a certain chemical in food, then persons eating that food could not be deficient, is being questioned.

Stress produces aging; an increased intake of vitamin E makes it possible for rats to withstand the aging process due to increased stress. It has been discovered that the aging process produces pigmentation within the cells of both animals and humans.

LITERATURE CITED

BOOKS

- Abdellah, Faye G., and Eugene Levine. Better Patient Care Through Nursing Research. New York: Collier-Macmillan Limited, 1965.
- Boder, Elena. Current Pediatrics Therapy. Edited by Sidney S. Gellis and Benjamin M. Kagan. Philadelphia: Saunders, 1966-1967.
- Cantin, M., Jean-Mare Diendonne, and H. Selye. "Effects of Vitamin E on Cardiomuscular Calciphilactic Lesions," Exper. Med. and Surg. 1962, pp. 318-320, cited by Herbert Bailey. Vitamin E: Your Key to a Healthy Heart. New York: ARC Books, Inc., 1964, pp. 94-96.
- Cantrow, Abraham, and Bernard Schepartz. Biochemistry. Philadelphia: W. B. Saunders Company, 1954.
- Caplan, Gerald. An Approach to Community Mental Health. New York: Grune and Stratton, Publishers, 381 Fourth Ave., 1961.
- Carpenter, Edmund, and Marshall McLuhan. Explorations in Communications. Boston: Beacon Press, 1960. (paperback edition, 1966)
- Christ, J. F. "Nerve Supply, Blood Supply and Cytology of Neurohypophysis," The Pituitary Gland, Vol. III. Edited by G. W. Harris and B. T. Donavan. Los Angeles: University of California Press, 1966.
- Cooley, Donald G. Modern Medicines. New York: Pocket Books, Inc., A Giant Cardinal Edition, 1963.
- _____. Dorland's Illustrated Medical Dictionary. 24th edition. Philadelphia, London: W. B. Saunders Company, 1965.
- Evans, Herbert McLean. "The Relations Between Fertility and Nutrition," Lectures on Nutrition, Mayo Foundation Lectures. Philadelphia: W. B. Saunders Company, 1924-1925.
- Fitzpatrick, Elise, Nicholson J. Eastman, and Sharon R. Reader. Maternity Nursing. 11th edition. Philadelphia, Toronto: J. B. Lippincott Company, 1966.
- Goth, Andres. Medical Pharmacology Principles and Concepts. Fourth edition. St. Louis, Mo.: C. V. Mosby, 1968.
- Green, David E., and Robert F. Goldberger. Molecular Insights Into The Living Process. New York, London: Academic Press, 1967.
- Griffen, John Howard. Black Like Me. Boston: Houghton Mifflin Co., 1961.

- Gelloin, H. V. "Carcinogens, Enzyme Induction and Gene Action," Advances in Cancer Research. Vol. 10. Edited by Alexander Hardow and Sidney Weinhouse. New York, London: Academic Press, 1967.
- Gerard, Ralph W. Food For Life. Chicago: University of Chicago Press, 1952.
- Harris, Ieuan. "The Chemistry of the Intermediate Lobe Hormones," The Pituitary Gland. Berkeley and Los Angeles: University of California Press, 1966.
- Hawk, Philip B., Bernard L. Oser, and William H. Summersen. Practical Physiological Chemistry. 13th edition. New York: The Blakiston Company, Inc., 1954.
- Hickman, Cleveland P. Gray's Encyclopedia of the Biological Sciences. Editor, Peter Gray. New York: Reinhold Publishing Corporation, 1961.
- Hilway, Tyrus. Introduction to Research. Second edition. Boston: Houghton Mifflin Company, 1964.
- Holman, Ralph T. The Vitamins. Edited by W. H. Sebrell, Jr., and Robert S. Harris. New York: Academic Press, Inc., 1954.
- Keele, Cyril, and Eric Neil, with the collaboration of John B. Jebson. "The Vitamins," Samson Wright's Applied Physiology. 10th edition. New York, London, Toronto: Oxford University Press, 1961.
- Krehl, Willard A., and J. J. Barboriak. Chapter 13, "Factors Affecting the Utilization of Food Nutrients by the Normal Organism," Nutritional Evaluation of Food Processing. Edited by Robert S. Harris and Harry von Loesecke. New York, London: John Wiley and Sons, Inc., 1960.
- Latham, Helen C., and Robert V. Heckel, with the collaboration of Margaret Moore. Pediatric Nursing. St. Louis, Mo.: The C. V. Mosby Company, 1967.
- Lerner, Aaron B. "Possible Physiological Function of Intermediate Lobe Hormones in Mammals," The Pituitary Gland. Vol. III. Editors, G. W. Harris and B. T. Donavan. Los Angeles: University of California Press, 1966.
- Lerner, Aaron B. "Assay of Intermediate Lobe Hormones," The Pituitary Gland. Vol. III. Editors, G. W. Harris and B. T. Donavan. Berkley and Los Angeles: University of California Press, 1966.
- Margilethe, Andrew M. Current Pediatrics Therapy. Edited by Gellis and Kagan. Philadelphia: Saunders, 1966-1967.

- Pfieffer, John. The Cell - Life Science Library. New York: Time Incorporated, 1964.
- Proudfit, Fairfax T. Revised by Coriane H. Robinson. Normal and Therapeutic Nutrition. 12th edition. New York: The Macmillan Company, 1961.
- Runyon, Richard P., and Audrey Haber. Fundamentals of Behavioral Statistics. Reading, Massachusetts, Palo Alto, London: Addison-Wesley Publishing Company, 1967.
- Russo, John Paul. "... Take As Directed." Edited by F. E. Shideman. Cleveland, Ohio: CRC Press, 1967.
- Sebrell, W. A., and Robert Harris. The Vitamins. Vol. II. New York: Academic Press, 1954.
- Selye, Hans. The Adaptation Syndrome. Palo Alto: J. M. Stacey, 1952. (Preface)
- Selye, Hans. The Stress of Life. New York: McGraw-Hill Book Company, 1956.
- Shafer, Kathleen N., Janet R. Sawyer, Audrey M. McCluskey, and Edna L. Beck. Medical-Surgical Nursing. Third edition. St. Louis: C. V. Mosby Company, 1964.
- Stewart, Dorothy M., and Pauline Vincent. Editors of Public Health Nursing: A Book of Readings. Dubuque, Iowa: Wm. C. Brown Company, 1968.
- Turney, Billy L., and George P. Robb. Simplified Statistics for Education and Psychology. Scranton: International Textbook Company, 1968.
- Wingstrand, Karl Georg. "Microscopic Anatomy, Nerve Supply and Blood Supply of the Pars Intermedia," The Pituitary Gland. Vol. III. Edited by G. W. Harris and B. T. Donovan. Berkeley and Los Angeles: University of California Press, 1966.
- Wooley, D. Wayne. Neurophysiology and Emotion. Edited by David G. Glass. New York: The Rockefeller University Press, and the Russell Sage Foundation, 1967.

PERIODICALS AND REPORTS

- Adams, C. R. A Report at the 19th Annual Proceedings of the Montana Nutrition Conference for Feed Manufacturers, Dealers, Salesmen and Livestock Men. Montana State University, Bozeman, Montana. February 5-7, 1968.
- Bhamerapravati, Natth, Sup Na-Na Korn, Prawase Wasi, and Sood-sa Korn, Tuchinda. "Pathology of Abnormal Hemaglobin Disease Seen in Thailand," The American Journal of Clinical Pathology, 47:6:745-759, 1967.
- Binder, H. J., D. C. Herting, V. Hurst, _____ Finch, and H. M. Spiro. "Tocopherol Deficiency in Man," New England Medical Journal, 275:1289-597, 1965.
- Binder, J. J., and Howard M. Spiro. "Tocopherol Deficiency in Man," The American Journal of Clinical Nutrition, 10:6:594-601, January, 1967.
- Blakeslee, Alton L. "News," Today's Health, October, 1963.
- Dicks-Bushnell, Martha W., _____ and Karen C. Davis. "Vitamin E Content of Infant Formulas and Cereals," The American Journal of Clinical Nutrition, 20:3, March, 1967.
- Cooley, Donald G. "Hormones: Your Body's Chemicals," Today's Health, 40:30, December, 1962.
- Cooley, Donald G. "What Is A Vitamin," Today's Health, 41:73, June, 1963.
- Dipalma, Joseph R. "The Pituitary Hormones: Potential Wonder Workers," RN, January, 1966.
- Graham, Don. "Selenium, Tricky Trace Element," Now, Montana State University, College of Agriculture, Bozeman, Montana, Fall, 1967.
- Harris, Phillip H. "Discussion, in a Symposium: Advances in the Detection of Nutrition Deficiencies in Man," The American Journal of Clinical Nutrition, 20:6:602-603, January, 1967.
- Horwitt, M. K. "Vitamin E in Human Nutrition--An Interpretative Review," Borden's Review of Nutrition Research, 22:1:13, January-March, 1961.
- Leonard, P. J., and M. S. Losowsky. "Relationship Between Plasma Vitamin E Level and Peroxide Hemolysis Test in Human Subjects," The American Journal of Clinical Nutrition, 20:8, August, 1967.

- Mattill, Henry A. "Vitamin E--the Tocopherols," Borden's Review of Nutrition Research, XIII:9, December, 1952.
- McHenry, E. W. "Nutrition and Endocrine Function," Borden's Review of Nutrition Research, XVI:3-4:20, March and April, 1955.
- Olson, Robert. "Are We Looking at the Right Enzyme Systems?" The American Journal of Clinical Nutrition, 20:6:609, June, 1967.
- Oski, Frank A., and Lewis A. Barness. "Vitamin E Deficiency: A Previously Unrecognized Cause of Hemolytic Anemia in the Premature Infant," The Journal of Pediatrics, 70:211-220, February, 1967.
- Prankard, M. D. "The Over-All Significance of Metabolism and Enzyme Activity in the Red Cell," The American Journal of Clinical Pathology, 47:3: 282-286, 1967.
- Quint, Jeanne C. "The Case for Theories Generated from Empirical Data," Nursing Research, Spring, 1967.
- Sebrell, W. H. Jr. "Changing Concept of Malnutrition," The American Journal of Clinical Nutrition, 20:6:653-658, June, 1967.
- Stowe, Howard D. "Alpha-Tocopherol Requirements for Equine Erythrocyte Stability," The American Journal of Clinical Nutrition, 21:2, February, 1968.
- Tappel, A. L. "Where Old Age Begins," Nutrition Today, 2:4:2-7, December, 1967.
- Tulloch, J. A., and N. K. Snood. "Vitamin E Deficiency in Uganda," The American Journal of Clinical Nutrition, 20:8:884-887, August, 1967.
- Weiking, Dorothy, and Kusum Verma, "A Histochemical Study of Embryonic Rat Liver in Avitaminosis E ¹, ², ³," The Journal of Nutrition, 91, 1, Part I:159, February, 1967.
- Wertz, J. M., C. R. Seward, E. L. Hove, and J. S. Adkins. "Interrelationship of Dietary Glycine, Methionine and Vitamin E in the Rat," The Journal of Nutrition, 94:2:129-138, February, 1968.
- Whitaker, Jo Anne, Eleanor G. Fort, Serene Vimokesant, and James A. Dinning. "Hematologic Response to Vitamin E in the Anemia Associated with Protein-Calorie Malnutrition," The American Journal of Clinical Nutrition, 20:7:789, July, 1967.
- Woods, Ruth. "The Essential Fatty Acids," Borden's Review of Nutrition Research, IX:4, April, 1948.

ABSTRACTS

- Adachi, Toru, Yukio Nakaji, and Masato Asahima. "Change of NAD Content in the Mouse Liver After Administration of A-tocopherol Nicotinate," (Nat. Inst. Hyg. Sci., Tokyo, Japan), Bitamin, 37(2), 143-6, 1968, (Japanese), Vol. 68, #66888f.
- Boguth, W., R. Repges, and M. Sernetz. "Thyroid Function in Rats Fed a Diet Deficient in Tocopherol and Ubiquinone," (Univ. Giessen, Germany), Int. Z. Vitaminforsch., 37(3), 287-292, 1967, (German), cited in Chemical Abstracts, Vol. 68, #20026v.
- Boguth, W., and M. Sernatz. "Thyroid Function of Rats on a Tocopherol-- and Ubiquinone--Deficient Diet," (Univ. Giessen, Germany), Int. Z. Vitaminforsch., 37(4), 412-415, 1967, (German), cited in Chemical Abstracts, Vol. 68, #66893d.
- Burke, C. "A Common Solvent Impurity Isolated...", (University of York, England), Journal of Chromatog., 31(1), 247-250, 1967, (English), cited in Chemical Abstract, Vol. 68, #10089d.
- Caravaca, Josefina, E. Grey Dimond, Sheldon C. Sommers, and Robert Wenck. "Prevention of Induced Atherosclerosis by Peroxidase," abstract cited in Science, Vol. 155.
- Carpenter, Stirling. "A Histochemical Study of Oxidative Enzymes in the Nervous System of Vitamin-E Deficient Rats," (Armed Forces Inst. of Pathology., Washington, D. C.), Neurology, 15(4), 328-332, 1965, (English), as cited in Chemical Abstracts, Vol. 63, #979g.
- Cawthorne, M. A., J. Bunyan, A. T. Diplock, Elspeth A. Murrell, and J. Green. "The Relation Between Vitamin A and Vitamin E in the Rat," (Walton Oaks Exptl. Station, Tadworth, England), British Journal of Nutrition, 22(1), 133-143, 1968, (English), cited in Chemistry Abstracts, Vol. 68, #47442q.
- Diamond, Ivan, and Rudy Schmid. "Oxidative Phosphorylation in Experimental Bilirubin Encephalopathy," cited in Science, Vol. 155, March, 1967, pp. 1288-1289.
- Dorodneva, V. I., and A. L. Shinkarenko. "Tocopherols and B-sitosterol from Walnut Oil," (Pyatigorsk. Farmatsevt. Inst., Pyatigorsk), Izv. Vyssh. Ucheb. Zaved. Pishch. Tekhnol., 1967, (Russian), (5) 101-104, cited in Chemistry Abstracts, Vol. 68, #14290k, 1968.

- Gedigk, P. "Formal Genesis of the Vitamin E Deficiency Pigment in the Electron Microscope Image," (University Marburg, Germany), Verhandl. Deut. Ges. Pathology, 47:258-260, 1963, (German), cited in Chemical Abstracts, Vol. 63, #3381g, 1965.
- Gortner, Ross A. Jr., and Joanne B. Ekwurtzel. "Incidence of Teratogeny Induced by Vitamin E Deficiency in the Rat," (University of Middletown, Conn.), Proc. Soc. Exptl. Biol. Med., 119(4), 1069-1071, 1965, (English), cited in Chemical Abstracts, Vol. 63, #18725d.
- Grau, R. "Effect of Tocopherol Supplementation on the Quality of Meat Products," (Bundesanstalt Fleischforsch., Kelkheim, Germany), Wiss. Veroeff. Deut. Ges. Ernaehr., 16:189-198, 1967, (German), as cited in Chemical Abstracts, Vol. 68, #10749u, 1968.
- Horwitt, M. K., et al. "Depot Fat Fatty Acids and Plasma Tocopherol Relationships in Man," (L. B. Mendel Research Laboratory, Elgin State Hospital, Elgin, Illinois), cited in Journal of Nutrition, 94:2:366-367.
- Larsen, Virginia L. "Stresses of the Child Bearing Year," American Journal of Public Health, 1:66:32, 1966, cited in Briefs, April, 1966.
- Lea, A. J. "Investigation of Adrenochrome as Cause of Schizophrenia," Journal Mental Science, 101:538-547, June, 1955, cited in What's New, No. 193, Abbott Lab, Chicago, Abstract #716.
- Loosli, Rolf. "Hereditary and Nutritional Muscular Dystrophy in an Inbred Strain of Mice," (Jackson Lab., Bar Harbor, Maine), Journal of Heredity, 56(2), 75-81, 1965, (English), cited in Chemical Abstracts, Vol. 63, #6071f.
- Merciai, V., F. Tozzi, and S. Bonclinelli. "Blood Lipid Behavior During Intravenous Administration of Lipifisan," (Terapia Chir, Univ. Studi, Florence), Acta Anaesthesiol. Suppl., 17(3), 19-35, 1966, (Italian), cited in Chemical Abstracts, Vol. 68, #20671b, 1968.
- Pototskii, I. I. "Effect of the Endocrine Glands on the Course of Eczema," (Med. Inst. Place., Kiev), Vrachebnoe Delo, (7), 90-94, 1965, (Russian), cited in Chemical Abstracts, Vol. 63, #12114b, 1965.
- Schmidt, H. E. "Determination of Tocopherols in Oils and Fats: Influence of the Tocopherols Content of Groundnut and Soybean Oils on the Course of Oxidation of These Oils by Heating," (Unilever Forschungslab., Hamberg, Germany), Fette, Seifen, Anstrichm., 69(12), 913-916, 1967, (German), as cited in Chemistry Abstracts, Vol. 68, #48421u.

- Stuyvesant, Wilfred V., and Weldon B. Jolley. "Anti-inflammatory Activity of d-a-Tocopherol and Linoleic Acid," (School of Med., Loma Linda University, Loma Linda, California), Nature, 216(5115), 585-586, 1967, (English), cited in Chemical Abstract, Vol. 68, #11566a.
- Tsagareli, Z. G. "Structural and Histochemical Alterations of the Nervous System as a Result of Hunger," Tr. Inst. Eksperim. Morfol., Akad. Nauk Gruiz, SSR-11, 51-9, 1963, (Russian), cited in Chemical Abstract, Vol. 63, #977f, 1965.
- Ulasevich, I. I. "Influence of Water-Soluble Analog of Vitamin K (vica sol) on Changes in the Sarcoplasmic Proteins of Muscles Developing in Vitamin E Deficiency," Byul. Eksperim Biol. i. Med., 59(7), 54-6, 1965, (Russian), cited in Chemical Abstracts, Vol. 63, #12178d, 1965.
- Van Woert, M. H. "Oxidation of Reduced Nicotinamide Adenine Dinucleotide by Melanin," (Med. Div., Oak Ridge Assoc. University, Oak Ridge, Tenn.), Life Science, (Oxford) 6 (24), 2605-12, 1967, (English), cited in Chemical Abstracts, Vol. 68, #65781d, 1968.
- Wertz, J. M., C. R. Seward, E. L. Hove, and J. S. Adkins. "Interrelationship of Dietary Glycine, Methionine and Vitamin E in the Rat," cited in Journal of Nutrition, Vol. 94, #2, February, 1968.
- Wozniak, Jan. "Effects of Ionizing Radiation on Pests in Cereals--Effects of Irradiation on Vitamin Content of Flour," (Pantw. Zakl. Hig., Warsaw), Roczniki Panstwowego Zakladu Hig., 16(2), 183-188, 1965, (Polish), cited in Chemical Abstract, Vol. 63, #13931b, 1965.