APPLICATION OF COMPUTER IN THE DETERMINATION OF PRIMARY STRUCTURE OF RED CELL PROTEIN - HAEMOGLOBIN IN HUMAN

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SUBMITTED TO THE DEPARTMENT OF MATHEMATICS / COMPUTER SCIENCES FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA IN PARTIAL FULFIMENT OF THE REQUIREMENTS FOR THE AWARD OF A POST – GRADUATE UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA.

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CERTIFICATION

This is to certify that this project is an original work carried out by me and has been prepared in accordance with regulations governing the preparation and presentation of project in the department of Mathematics / Computer Science, School of Science, Federal University of Technology, Minna.

DR. YOMI JAYESIMI
PROJECT SUPERVISOR

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MR. L. Z. EZEAKO
HEAD OF DEPARTMENT

SIGN

EXTERNAL EXAMINER

SIGN

DEDICATION

Glory be to God; the king of kings; the Alfa and omega. The beginning and the end; who gave me this opportunity to start this course and finish without no problem. This project work is dedicated to my parents; late Dorcas Awero and Mr. Thomas Olanamu who stands and coverage during various difficulties of life serve as enduring confidence and challenge to move forward in the faces of threatening impossible circumstance, their moral advice, to make us number one in the committee of equals and all round excellent in our various pursuit serve as sources of untiring inspiration and lasting happiness to all their fruit.

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ABSTRACT

Every living cell needs both oxygen and nutrients (food) to be maintain and sustain life. The need is met in unicellular organism simply by exchange (diffusion) of air between the organism and its immediate environment. However, with the complexity of the multicullular organisms, the simple exchange is not only insufficient but also impossible thus there is development of specialize physiologic systems to meet the responsibilities of all physiologic functions and needs of the organism. To meet the need of air and nutrients for each cell, the circulatory and respiratory system (of which blood especially in vertebrates is a vital component) play the central role.

Thus, any deficiency of the components of these system would lead to attered physiologic functions which may threaten the life of the organism. For instance, the change in normal structure of oxygen carrier protein – haemoglobin would have detrimental effect on the functions of the Red cell protein. This situation is evidenced in various molecular abnormalities of haemoglobin structure, which thus result in haemoglobinopathesis of which sickle cell anaemia is one.

A studious look into the molecular structure of haemoglobin in relation to its functions forms the bases of structure / function analysis which is on eye – opener to various familiar diseases. In this study, various changes in structure of haemoglobin with their correlating pathological consequences are elucidated as genetic molecular disease. Genetic disease defile the solution of therapy, consequent upon this, is the genetic education counseling given not only to

relieve the symptous of the inborn haemoglobin related diseases, but also aims to reduce it, if not totally eliminate the occurrence of genetic disease and victim of such, resulting from genetic doom.

TABLE OF CONTENTS

Chapter Four Outputs of the programs:-

CHAPTER ONE

1.1 Protein involved at the centre of action of bioprocesses and form the major class of biologic macromolecules. It has defined physiochemical properties and therefore it is generally easier to isolate and characterize than other biomacromolecules such as lipids, polyssacharides and Nucleic acids. It help in body building in terms of cell growth, replacement of dead cells and repair of damage cells. Cells are made up largely of protein in relates of the functions it performed. Protein perform two important functions: e.g Dynamic and structural functions.

Under structural functions, protein form the matrix of bone, fragment and provision of structural strengths, elasticity and vascular system to the organism e.g alpha-keratin has essential structural role in epidermal tissue. Also under dynamic function of protein include catalysis of chemical transformation, metabolic control, contraction and transport. These functions can be summarized are follows: Control and regulatory protein in translation and transcription. Histones associated with DNA (Deoxy - Nucleic Acid), receptor, and exchanged proteins which control genetic expression. Ribosomal protein in translation prcess roles in contractile mechanisms–myosin and actin that function in muscle contraction. Transport proteins–Haemoglobin in blood, myoglobin in muscle transport respiratory gases. The transfer is concerns with transport of Iron II (Fe^{2+}) etc. Hormones e.g Peptide hormones such as Adreno Corticotrophin, Calcitonin, Glucagon, Antidiuretic hormone, follicle stimulating hormone, luteinishing hormones, insulin, thyrotropin in blood, and somatotropin

hormone. Thyrotropin releasing factor, Methionine enkelphadin. (Copiate–like peptide in the brain which inhibit sense of pain). Little gastrin–hormone which stimulates parietal cells to secret aid. Vasodilator peptide–plasma body. Substance P–which serves as chemoneuro transmitter. Protective proteins–immunoglobulins and interferons which act against bacterial and viral infection respectively. Fibrin stops loss of blood on injury to the vascular system.

Apart from above mentioned, protein also perform underlisted functions:

(1) It transport and store biologically important substance e.g mettations, oxygen, glucose, lipids, and other molecules.

(2) Immunoglobulins proteins of immune systems form an essential biodefence system in tigher animals

(3) Protein are the active elements and product of the expression of genetic information, the Nucleic acid are for the most part information banks upon which protein act.

(4) protein form muscle fibre and other contractile assemblies. Protein generate and co-ordinate mechanical motion of numerous bio-responses including separation of chromosome during mitosis and eye movements (Rhodopsin acquires sensory information that is processed through the action of nerve cell proteins).

(5) It serve as regulators of these reactions both directly as components of enzymes and indirectly in the from of chemical messengers such as hormones and receptors of hormones.

(6) Protein functions as enzymes that catalyse the complex set of chemical reactions that are collectively referred to as life.

(7) It is passive but structural role-Collagen which produce bones tendons, and ligaments with their characteristic tensile strength, such structure e.g hair, nail were made up of essentially protein. As such protein are the building blocks of life.

Proteins are liner polymers of amino acids, which are integral submits of polypeptides and proteins. Many amino-acid and their derivatives are of biochemical importance, these alternative specialize role of amino-acids. Beside these roles performed by protein. It also has biological important functions such as

(a) Amino acid and derivative of communication between cells e.g glycine, neurotransmitter (sustenance released by nerve cells to alter their neighbours).

(b) Thyroxine – an Iodine containing thyroid hormone stimulates vertebrate metabolism.

(c) Amino acids are intermediates in various metabolic processes citruline which is intermediate in urea biosynthesis. Homocysterine is intermediate in amino acids metabolism.

(d) S- a denosyl methionine serve as a biologic methylatiny agent.

(e) Histamine serve as a local mediator of allergic reactions.

(f) Almost 250 different amino acids have been found in plants and fungi, most of them being toxic suggests that they protective function. Infact,

some of them are medical useful antibiotics e.g Azaserine, B-cyamoalaline etc, L–amino acids are present in proteins but D–Amino acids are present in many organism. They serve as constituents of bacterial cell walls, where they serve as defensive function. D – Amino acids render the bacterial wall less susceptible to attack by the peptidases that D – Amino acid occur as component of many antibiotics including Valiomycin, Actinomycins, Gramiadins. Thus amino acids and their derivatives also have wide independent biologic roles as neurotransmitters, metabolic intermediates and poisons.

(h) Apart from all these, amino acids are also energy metabolites and many of them are essential nutrients.

AMINO ACIDS FOUND IN PROTEIN

No	NAME	A-NAME	PK_A	PK_B	PK_R	I_P	RH
1.	ALANINE	ALA	2.3	9.9	-	6.00	0.5
2.	HISTIDINE	HIS	1.8	9.1	6.0	7.59	0.5
3.	GLUTAMIC ACID	GLU	2.2	9.9	4.3	3.25	-9.9
4.	ASPARTRIC ACID	ASP	2.0	10.0	3.9	2.77	-7.4
5.	LYSINE	LYS	2.2	9.2	10.8	9.74	-4.2
6.	CYSTEINE	CYS	1.8	10.8	8.3	5.07	2.8
7.	TYROSINE	TYR	2.2	9.1	10.1	5.66	2.3
8.	GLYCINE	GLY	2.3	9.8	-	5.97	0
9.	VALINE	VAL	2.3	9.7	-	5.96	1.5
10.	LEUCINE	LEU	2.3	9.7	-	5.98	1.8
11.	SERINE	SER	2.2	9.4	13	5.68	0.3
12.	THREONINE	THR	2.1	9.1	13	5.60	0.4
13.	TRYPTOPHAN	TRY	2.4	9.4	-	5.89	3.4

14.	PROLINE	PRO	1.9	10.6	-	6.30	3.3
15.	ISOLEUCINE	ISO	2.3	9.7	-	6.02	2.5
16.	PHENYLALANINE	PHE	2.6	9.2	-	5.48	0.4
17.	METHIONINE	MET	2.2	9.3	-	5.74	1.3
18.	ASPARAGINE	ASN	2.0	8.8	-	5.41	-0.2
19.	GLUTAMINE	GLN	2.2	9.1	-	5.65	-0.3
20.	ARGININE	ARG	1.8	9.0	12.5	10.76	-11.2

A – NAME	ABRREVIATED NAME	
PKA	PK OF ALPHA CARBOXYLIC	(COOH group)
PKR	PK OF ALKYL (R) SUBSTITUENT	(ALKYL group)
PKB	PK OF ALPHA AMINO (NH2)	(AMINO group)
RH	RELATIVE HYDROPHOBICITY IN KCAL /MOLE	
IP	ISOCELECTRIC POINT	

Relative hydrophobicity kcal/mole measured by the distribution of the amino acids between a non-polar solvent either ethanol or Dioxane water, negative values indicate preference water and positive value indicate a preference for non-polar solvent. The data with astericks are calculated values. Amino acids with double astericks are aromatics.

The specific chemical and biological properties of an individual protein should find explanation in term of an exact description of its structure in the native state. Some deficiencies of protein in pathological condition could be traced to alter structure of such protein fir example sickle ceil anaemia arisens from the altered structure in the position 6 of B chain of haemoglobin. The only point substitution of water soluble amino acid (glutamic acid) by lipophiltic amino acid (valine) in the B6 position of haemoglobin chain results in deadly

sickle cell disease. This single intermolecular change so alters the properties of the haemoglobin molecule that anaemia and other defects are produced.

The functions of protein biologic system to maintain life are myriad in which several biologic life depend. When there is an equilibrium shift of the intricate normal balance either in function or quantity, these results a consequential abnormality which may be mild or severe. The mild situations may be life compatible showing no pathological symptoms, or may pose constant threat to life disease. The defect either physiological or biochemical produced by abnormal or subnormal protein may be due to alteration in the structure of the protein. This is specially if such structural variant occur at the active site of the protein or at the active site dependent portion usually refered to as allosteric site. A curious study of the effect of altered structure of such protein in relation to its function would lead to knowledge of the structure function relationships of the protein molecule.

For haemoglobin, other equally clinically important and less severe clinical defect commonly referred to as haemologlobino pathies do exist and arise from alteration of the primary structure of haemoglobin by substituting of the composite amino acids at one position or other with other amino acids.

1.2 OBJECTIVES OF THE STUDY

Sickle cell anaemia do not occur in African, about 7-10% people lives in United State of America carry sickle cell trait (character). This disease, called sickle cell anaemia is rare about 0.3% to 2.5%. Simple stastical revealed that the sickle cell anaemia also occur in African, thus proper awareness about and

tangible knowledge of the disease is necessary with a view to reducing the genetic transmission of the disease. Evident also show that sickle cell anaemia through clinical processes that it is incurable and fatal, ccupled with only the clinical possibility of treating the clinical symptoms rather than the disease. Proper, evoke a social need to guide against the chance of giving rise to sicklers who will spend their short span lives in the menance of excruciation pain. The erroneous belief that sicklers hardly live more than a few years after birth, can also be corrected. Given proper medical care and life supporting healthy environment, many a sickler has been witnessed to lived out his full life span. Other important points is that sickling is neither the fault of the mother, father nor the child, however it is an accumulation of genetic errors from both parents which gives rise to the disease in their child. Thus there is much need for proper genotype check up to ensure marital compatibility prior to wedlock; in order to avoid the unfortunate incident.

1.3 THE SCOPE OF THE STUDY

Haemoglobinopathies belong to a class of blood diseases among many, they involve in abnormal conditions of the corpuscular elements of the blood – the red blood cells. Other abnormal conditions involved the leucocytes or white blood corpuscles or cells and the platelets and the tissues in which they are formed at the bone marrow, the lymph nodes, the spleen which are often referred to as a haemotopoietic system.

The scope of study is restricted to blood disease that involves the red cells of the blood, more specifically concerning the molecular disease arising

due to change in the structure of the transport protein haemoglobin of the red blood cell and how this change in structure affects the transport function of oxygen, that is the physiologic effect and the ultimate clinical consequence.

1.4 THE TISSUE FLUID – BLOOD

Blood is a cell containing fluid that transport oxygen, water, corbondioxide, products of metabolism and internal secretion e.g. hormones. The blood and lymph are the important fluids connecting the diverse anatomical structures of the mammalian organisms. Blood being a tissue that constantly circulate throughout animals, serve as means by which constancy of the internal environment is maintained. It is the route by which the defense against the injury and disease may be quickly mobilized.

Blood in mammals is the red fluid that is pumped by the heart into arteries and returns to the heart through veins following a complex but completely closed circular path. The red colour of the blood is imparted by the porplysin ring of haemoglobin – an oxygen carrying protein red cells. The specific gravity of human blood ranges between 1.055 and 1.065. Its viscosity is approximately 5 to 6 times than that of water. The total volume of blood in vascular system approximately 80% of body weight and about 5-6 litres of blood in an adult physiologic man. Infants do have a large blood volume per body weight than adults do.

In an adult man, about 5.4 million red cell per ml are present while in adult women, the red cells count is less, about 4.8 million red cells per ml. In infants, red cell count is more at neonatal stage than that of adult woman, but

as the baby grows, the number of red cells decrease until it is lesser than of woman. The difference in content of red cell counts of male and female babies began to be noticed at puberty. This perhaps is due to different physiologic effects of hormones produced by both sexes at puberty.

1.5 COMPOSITION OF THE BLOOD THE PLASMA

1. The liquid part of the blood is the nearly colourless plasma in which are dissolved various solute constituents. The various plasma solute are: protein 7% inorganic salt approximate 0.9% remainders are diverse organic compounds other than protein.

i) Non protein organic compounds of blood are mostly: (i) Metabolites normal range in mg/dl such as glucose: 65 – 90. Fructose: 6-8, pentose: 2-4 amino acids 35-65. (ii) Bye – products of metabolic pathways such as: urea 20-30, Bilirubin 0.2-14. creatinine 0.2–0.9, creatinine 1-2, uric acid 2-6.

2. CARBOHYDRATE:- Macromolecules such as glycogen 5-6 polyssacharides as hexoses 70-1-5, glu-cosanine 60-105 hexuronates 0.4-1.4.

3. ORGANIC ACIDS:- These are mainly all intermediates of tricarboxylic acid cycle or kreb's cycle viz citric acid 1.4-3.0 alpha ketoglutaric acid 0.2-1.0, Malic acid 0.1-0.9, succinic acid 0.1 – 0.6, acetoacetic acid 0.8-2.8 and others are lactic acid 8-17 and pyruvic acid 0.4-2.0.

4. LIPIDS:- The total lipids ranges between 285-675 mg/dl within the plasma. The major ones are cholesterol 130-260, Neutral fat 80-240, Esters 90-190. Total fatty acids 150-500 and others.

5. The plasma protein ranges in concentration normally in normal human body of adults from 5.7 to 8.0g/dl. The protein which can be fractionated by electrophoresis have majors fractions as

i. Albumin 54-58% involved in ostnotic regulation, transport of fatty acids, billirubin and aldosterone.

ii. Alpha-globulins 6-9% involved as protein in coagulation of blood and as protease inhibitors.

iii. Fibrinogen 2.5-5.0% - chiefly responsible for the formation of blood clot during haemostasin.

iv. Cryoglobulins-Implicated during inflammatory disease of rheumatoid arthritis, multiple myeloma etc.

v. Betha II-globulin 8-9%, Bi-globulins 13-14% as lipoproteins.

vi. Betha II-microglobulin-associated with HL-A histo compatibility Antigen complex which is implicated in graft rejection, autoimmune reactions etc.

The plasma solutes being soluble materials apart from the specialized physiologic roles they play function in osmotic regulation of the blood. Their osmo-active nature helps in maintaining normal water balance between the blood and the interstitial fluid. The excess water and poisonous by-products of metabolism e.g urea, uric acid, etc are passed to the kidney for excretion. Also some of the blood plasma solutes are used as detoxifying and conjugating

agents for various endogenously produced poison and xenobiotics-foreign chemical agents. Their functions in this wise form a hall mark of elimination of drugs and poisons as well as endogenous excretable products from the body. This is utmost importance especially in the elimination of highly lipophyllic agents from the body system as well as reducing its activity and toxicity.

Of considerable clinical and physiological importance are the roles played by blood plasma solute in the maintainance of blood viscousity and density within the normal range. These are vital to maintanance of normal and sufficient systolic pressure which allows for blood circulation round the body. The body defence mechanism through the agents of antibodies (immunoglobulms) is up held by globin serving as precursor in the biosynthesis of antibodies.

ii. **THE CELLULAR COMPONENTS OF BLOOD**

The living components of the blood are the cellular parts, they perform specific functions which are crucially important to the well-being and existence of the body as a whole. They are white blood cells, red blood cells; thromobo cytes or platelets.

(A) **THE WHITE BLOOD CELLS OR LEUCOCYTES**

They are the largest cells among the cellular component of blood, they are nucleated and make up to 4500-11000 cells per ml of blood (white blood cell count) in normal ranges. It comprise of three different cells; gramulocytes, lymphocytes, and monocytes. Fluctuations occur in white cell count during the

day, whilst lower values are obtained at rest and higher values during exercise. Violent, physical exercise may cause the count to exceed 20,000.

Granulocytes are larger than red blood cells, they have multilobed nuclei and contain large number of cytoplasmic granules. They may be present for few hours in circulation and then distributed throughout tissue spaces in large number. Granulocytes make up 50-65% of the white cell total and are distinguished into three classes based on their reactions with neutral, basic and a acid dyes. Neutrophills makes up to 3000-5,500 cells per ml of blood in an adult or two third of all the white blood cells. They are actively phagocytic, engulfing bacterials and other fine particles and they may destroy living microorganism. Granule of neutrophillis contain potent enzymes capable of digesting many types of cell materials.

Eosniophills are of 50-100 cells per ml of blood in an adult or 1-4% of the total white cells. They are actively phagocytic and are involved in tissue oxidation in which there are antigen antibody interactions.

(iii) Basophills make up to 0-40 cells per ml of blood or up to 1% of white cell count.

B LYMPHOCYTES: They are about 2000-3000 cells per ml of blood in an adult or about 28-42% of all white cells. They have single round nucleus each, slightly larger than red blood cells, the nucleus occupies most of the cells. They are found in large numbers in bones, spleen, tensils, they enter circulation through the lymphatic channels Lymphocytes are concerned with vital defence mechanisms pertaining to acquired

immunity to foreign cell antigens. They are responsible for immunologic reactions to invading organisms and to foreign cells e.g transplanted organs or cancer. Also responsible for immunologic reactions to foreign protein and other antigens not necessarily derived from living cells.

There are two classes of lymphocytes namely;

Class (1) Contain cells which produce immunoglobulnis including various types of antibodies in response to stimulation by an antigen. Class (ii) Contain cell which are concerned with cell-mediate immunity. These lymphocytes participated in the rejection of transplanted tissue and involved in certain types of allergy. They present important protective mechanism that prevents the proliferation of foreign cells, within the host, but it is also the mechanism that makes organ transplantation difficult. Rejection of a tissue graft is largely accomplished by cell-mediated immunity. Lymphocytes migrate to the area of the graft and cause its destruction. Cell mediated immunity is also involved in other reactions including the tuberculin reaction.

As such, in immunologically competent individuals white blood cells confer immunity against intruding antigens and defence of the body against unwanted exogenous and endogenous agents.

(c) MONOCYTES:- These are 300-700 cells per ml of blood or 4-8% of white cell count. They are characterized by lobulated nucleus with rounding projections. They are largest of all the white blood cell, their cytoplasms containing fnie granules. They are actively phagocytic, actings as scavengers and they are found in inflamed tissue earlier than graunlocytes. They are found

at sites of chronic infection where they are involved in ingestion of infections agents as well as red cells and other large particles. Monocytes are also precursors of the large phagocytic cells of the tissue called the macrophages.

THE PLATELETS OR THROMBOCYTES

They are nucleated and incapable of cell division, they are adhere to each other but not to cells or white blood cells. The normal count ranges between 150,000 and 300,000 cells per ml blood. Their functions is related to haemostastis which is the prevention and control of bleeding. If thrombocyte are not present haemostastis will not occur, and protracted bleeding from small wounds would occur and prolonged bleeding time results. The normal resistance of capillary membranes to leakage of red blood cells is dependent upon platelets. Also platelets contribute substance essential for the normal coagulation of the blood and they cause striking or retraction of a clot after it has been formed. Platelets are formed in the bone marrow, but the spleen serves as their reserve store.

UNIQUE PROPERTIES OF BLOOD

CHARACTERISTICS OR PROPERTIES	RANGE OF NORMALITY	CHEMICAL COMPONENT	RANGE OF NORMALITY
Volume measurement	7 – 9% body weight	Blood Glucose	80 – 120
Specific Gravtiy 25^{0c}	1.05 – 1.06	Serum Calcium	8.5 – 11.5
Volume Index	0.9 – 1.1	Blood Protein Bound Iodine	4.0 – 8.59
HB Content	14 –16g/100ml	Blood urea nitrogen (BUN)	8.0 – 20
Bleeding Time	1 – 31 minutes	serum sodium	312 – 342

8.	Colour Index	0.9 – 1.1	Serum Total Cholesterol	130 – 250
9.	PH (acid-Base Measurement)	7.35 – 7.45	Serum Protein total plasma	5.9 – 7.59
10.	Red Blood cell	$4.5x5.5x10^6/mm^3$	Albumin/Globulin	1:3:1-2.9:100
11.	White blood cell	5000-10,000/ml	Plasma Fibrinogen	290 –500
10.	Platelets	200,000-400,000ml	Blood non-protein Nitrogen (NPN)	24 – 40
11.	Coagulation Time	5.5 –12.5 minutes	Serum potassium	14-21
12.	Heamacrit	47-50%	Blood uric acid	3.5-5.0
13.	Prothrombin time	10-15 SEC		
14.	Relative viscousity 38^{0C}	4.7	Serum inorganic phosphorns	2.4 – 4.0
15.	Sedimentation rate	0 – 200mm first hour		

N.B Value except where indicated are in milligram per 100 milliliters. Haemocrit value: 42 – 54% of total value in men, and 37 – 47% of total value in women.

Valume of blood in an average person amounts to about 70ml (2.30z) for each kg (2.21b) of body weight. Viscosity compared to that of water has been formed, platelets are formed in the bone marrow, but the spleen serves as their reserve store.

(c) **THE ERYTHROCYIES OR RED BLOOD CELLS**

The human red blood cells are non-nucleated, biconcave disc with a diameter of a 6 – 9nm. Red cells plays transport and regulatory functions. The circulatory red cells and the total mass of erythropietic cells from which they are derived are term the erythron. The erythron, though dispersed organ has prime

functions of transporting of oxygen and carbon dioxide and maintenance of the PH (Acid-base concentration) of blood. The matured cells of erythron, the circulating red cells have known lifetime in circulation about 120days (3moths). During its life time, a red cells travds about 175miles through the circulation.

Erythrocyte is a highly specialized cells, its cytoplasm contain 34% solution of haemoglobin, completely devoid of subcellular organelles such as nucleus mitochondria lysosomes, ribosomes endoplasmic reticulum, Golgi bodies etc. The anucleated nature of red cells confers some functional advantage in that nuclei occupy space and require oxygen. These in mammals have most efficient red cells at least so far as oxygen-carrying ability is concerned. Red cells are thus is the centre, which perhaps increases the efficiency in gas transport.

The efficiency in gas exchange and transport may also be increased by the fact that the shape of red cell can be distorted in cones, clubs dumbells as they pass through extremely narrow blood capillaries. Erythrocyte behave as osmometer, swelling and strinking with increases and decrease in osmotic pressure of the medium red cell containing K^+, Na^+, Ca^{2+}, mg^{2+} as intracellular cations and the anions are cl^-, $HB^{\circ}_3^-$, HB, inorganic $P0^{2}_4^-$, 2,3 diphosphogly cerate. As red cells aged and are removed from circulation by the cells of reticulo-endothelia system, their haemoglobin HB is degraded. The globin of HB is hydrolysed to their amnioacid constituents, which are reutilized for protein synthesis, iron is transported by transferring to bone marrow and other tissues were reutilized for heme systhesis. The protoporphyrin from heme is degraded

in reticulo-endothelia cells and the liver and the resulting bile pigments are excreted via bile into intestinal lumen.

Dearrangement of any of these processes at any phases of the life cycle of the red cells can lead to severe human disorder. The altered rates of red blood cell (RBC) production and destruction as a result of abnormal condition ranging from malnutrition to hereditary defects may shorten the life span of the red cells giving rise to anemia and defective function of the erythron.

CHAPTER TWO

FUNCTION OF HAEMOGLOBIN

2.1 THE RED CELL PROTEIN – HAEMOLGOBIN

Human haemolgobin is a conjugate protein consisting of prosthetic groups (i.e non amino acid moietys) of hemes and four polypeptide chains which may be A, B, G, or D. Fetal haemoglobin consists two A – chains and two G-chains, while normal adult haemoglobin has two A-chains and two B-chains. The two A-chains are identical in composition and sequence, however, they are usually for the sake of identity reffered to as A_1 A_2 chains. The same thing is applicable to B chains, being refered to as B_1, B_2 chains. Each peptide chain is conjugated to a heme group ferropro-toporphyrm ix. The four polypeptide chain haemoglobin mesh together within the little space in the interior of red cells. The force linking the four chains involve only secondary forces such salt linkages and hydrophobic bonds. The protein part-globin is a basic and colourless protein joined to the heme group. The distal imidazole group of histidinc (HIS) at a position 63 in the B-chain and G chain or position 58 in the A-chain are in the direction to heme Fe^{2+} that has a space which is occupied by lipohillic oxygen. The hydrophocity of the heme pocket accounts for non-oxidation of Fe^{2+} to Fe^{3+} and that allows for reversible binding of oxygen. Also it places the heme in the environment of low dielectric constant /similarly imidazole group of His at position 92 in the B- chain bind's proximally to the heme Fe. In A-chain, His at position 87 is proximally bound to the heme Fe. The two proportionate side chain of each heme lie in juxtaposition to the

positive changed nitrogen's of a lysine and Arginine residue at position 82 and 104 of the B-chains.

In B-chain, there are 146 amino acids components. The possible numbers of different polypeptide chains that can be obtained with n number of amino acids is given as 20^n, since each amino acid had 20 different choice available. Thus, there are 20^{146} B-polypeptide chains. However, the number is tremendously reduced to few by the predetermination of the polypeptide chain sequence by the specific gene, coding for the chain. In the polypeptide chain, if the number of chiral centers is N (all biologic amino acids are asymmetric or chiral except glycine), then 2N different possible steroinomers and 2N-1-enantiomeric pairs of polypeptides chains that can be produced. The colour of the blood arises from the characteristic absorption spectra of the heme group (ferriprotorphyrin x) at the wavelength of red colour.

It is crucially important to note that, among the many B-chains for example, that are genetically determined, only the normal adult B-chain sequence is full compactable with normal physiologic functions of haemoglobin that maintain life.

2.2 FUNCTION OF HAEMOGLOBIN

(a) TRANSPORT FUNCTION OF HAEMOGLOBIN

In the lungs, the haemoglobin chains bind to oxygen molecules to one chain facilitates the binding to other chains in a red cells. At the same time, DPG binds to each chain or haemoglobin at the allosteric site. However binding 2,3 – DPG to oxy-Hb is much weaker than that of Deoxy-haemoglobin, this stablizes

deoxy-haemoglobin over oxygen haemoglobin. The physiologic implication of this is that, for oxygenation of deoxy-Hb, high oxygen tension is needed which is provided for, by high oxygen tension in the lungs, which completely saturate Hb. The physiological effect of DPG is upon the release of oxygen in the extra pulmonary tissue where oxygen tension are low. High DPG signifies the efficiency of oxygen delivery in the blood. Rise of DPG is noted in the red blood cells in conditions associated with tissue hypoxia- e.g. Anaemia, cardiopulmonary in sufficiency and high altitudes. The results in substantial increase in amount of oxygen delivery, because the venous blood returning to the heart of a normal individual at rest is at least 60% saturated with oxygen. This is possible if the PO2 (partial pressure of oxygen) in the lungs remain high enough that oxygen binding in the lungs is not compromised.

BOHR EFFECT

Bohr effect occur when the acidity of Hb increases as it binds oxygen. Bohr effect is equivalently in the increase in basicity of haemoglobin as it releases oxygen. Thus, low acidity/basicity weakens haemoglobin oxygen affinity enhancing oxygen delivery $HHb + O2 = HbO2 + H+$ (2.4.1)

Thus, increase in proton ion concentration (H+) with favour formation of free oxygen from HbO2 and conversely that oxygenation of Hb will lower the Hb to the solution carbondioxide is closely to Hb and to the problem of maintaining a constant PH in the blood. Carbon dioxide (CO2) is present in the blood in three major forms;- (i) Dissolved carbon dioxide (ii) As bicarbonate (HCO3)

formed by ionization of H_2CO_3 produced when carbon dioxide reacts with water.

$CO_2 + H_2O = H_2CO_3$ (2.4.2). (iii) As carbamino groups – CO_2 reacts with amino – NH_2 groups of protein. Each of these is present both in arterial and in venous blood. Carbon dioxide after it enters the blood stream for transport, generates hydrozonium ions H_3O^+ in the blood; through H_2CO_3 (Hydrogen carbonate) formation. Carbon dioxide (CO_2) entering the blood diffuses in to the erythrocytes. Within the erythrocytes, most of the CO_2 is acted upon by intracellular enzymes – carbonic anhydrase which catalyse the reaction.

$CO_2 + H_2O = H_2CO_3$ (2.4.3)

Because of the compartmentalization of carbonic anhydrate, essentially all of the conversion of CO_2 to H_2CO_3 and HCO_3 occurs inside the red cells. But most of the HCO_3 diffuses to the plasma, hence venous plasma bicarbonate is higher than arterial. The absence of carbonic-anhydrase in the plasma causes the taking up of carbon dioxide from the blood. This is done by the reaction of carbon dioxide with amino group of protein with red cells to form carbamino group. Haemoglobin protein is most important in the reaction. The deoxy-Hb forms carbamino –Hb more readily than oxy-Hb does, and oxy Hb causes the release of CO_2 bound in carbamino –Hb. Thus, deocy-Hb by binding CO_2 in the tissues where CO_2 tension is high, carries the gas to the lungs, where binding of oxygen to haemoglobin causes the releases of CO_2 to the atmosphere.

The formation of a carbamino group is like HCO3 formation a process that generates H+ carbamino groups can be formed only by uncharged amino groups in the Hb protein, this limit the number that potentially participate in this reaction. Essentially, only the 4 terminal amino groups of Hb chains can form carbamino product. The N –terminal amino group of B – chain forms part of the binding site of DPG thus competition arises. Carbon dioxide dimishes the effect of DPG and DPG in terms diminishes the ability of Hb to form carbamino Hb, thus the releases of carbon dioxide. Essentially, DPG has a releasing effect on oxy-Hb to release oxygen in the tissue where oxygen tension is high. Conversely, on carbamino –Hb to release carbon dioxide to the atmosphere in the lungs where carbon dioxide tension is low. Also in the tissue, with high tension of CO2 the H+ is high, leading to the release of oxygen form oxy-Hb and in the lungs the H+ concentration is low i.e. basicity high, leading to Hb to form oxy-Hb.

B. REGULATION OF BLOOD PH

Haemoglobin also plays the major role in handling the H^{+}_{30} ion (acid) produced by carbon dioxide transport. Haemoglobin has 38 Histidine residue per tetramer, these therefore provide the bulk of Hb's buffering capacity. In the whole blood, Hb buffering absorbs about 50% of the acid generated in the normal carbon dioxide transport. The buffer system (the inorganic phosphate buffer the plasma protein buffer and haemoglobin buffer) minimizes the change in PH that occurs when acid base is added to the blood. This controls is necessary to prevent change in intracellular PH which in turn may profoundly

affect metabolism. Also, protein conformation, essential for activity, is affected by change in PH and thus affects enzymes activity.

PHYSICAL FACTORS THAT AFFECCTS HAEMOGLOBIN

OXYGEN BINDING TEMPERATURE

High temperature weakens Hb's oxygen affinity. Temperature has significant effect on oxygen binding by Hb. At below normal temperature, the binding is lighter resulting in increase percentage Hb needed for saturation, thus low partial pressure PO2 is needed for saturation. At high temperature the binding is weaker, thus higher for is needed for saturation. High temperature and high level DPG have enchacing unloading of oxygen. The temperature effect is physiologically useful, as it makes additional oxygen available to support the high metabolic rate in exercising muscle and in fever with elevated temperature. The relative insensitivity to temperature of Oxygen binding at high PO2 minimizes compromise of oxygen uptake in the lungs under these conditions. The lighter binding of oxygen which occurs in hypothermic conditions is significant in hypothermia induced for surgical purposes. The decreases oxygen utilization by the body, and increase solubility of oxygen in plasma at low temperature, with the increases solubility of carbon dioxide, which acidifies the blood compesate for Hb's diminishes abilitys to release oxygen.

(2) PH

Low PH weakens Hb's oxygen affinity. Low PH (acidity) enhance oxygen delivery whereas high PH (basicity) increases binding of oxygen saturation of

Hb with oxygen increases with increase in PH. The influence of PH upon oxygen binding is physiologically important, since a decrease in PH signifies increase in oxygen demand. An increased metabolic rate results in increased production of carbon-dioxide and as in muscular exercise. Lactic acid also is produced by hypoxic tissue, these acid produced by metabolism help release oxygen to support that metabolism. The increase in acidity of Hb as it binds oxygen is known as the Bohr effect or Bohr effect is the increase in basicity of Hb as it release oxygen.

2.3 HAEMOGLOBINPATHIES

These are many inherited abnormalities of Hb synthesis in which there is formation of a structurally abnormal haemoglobin. They may involve in the substitution of one amino acid in one type of polypeptide chain for some other amino acid, or they may involve absence of one or more amino acid residues of a polypeptide chain or abnormal duplication at oxygen binding sites, heme pocket inter-chain contact, an allosteric sites, salt bridges and other functionally important locations are always critical resulting in change in physico-chemical properties and corresponding change in activities etc which reduce the efficiency of haemoglobin as oxygen carrier.

Some abnormal Hbs have altered affinity for oxygen, if oxygen affinity is increased (P50 decreased), oxygen delivery to the tissue will be diminished unless some sort of compensation occurs. Example Hb Ramier has P_{50} of 12.9mm Hg about 14Mm hg lesser than that of normal adult haemoglobin Hb_A 27mm Hg. The body of person with Hb Ramier responds by producing more

red cells polycythemia and more Haemoglobin. In Hb chesa peake, there is also increase affinity for oxygen and thus decreas delivery of oxygen to tissues at low oxygen tension. Polycythemia is the body's response to the defect. Methemoglobinemia ni which case abnormal amount of methemoglobin is in the blood. Methemoglobin is a form of haemoglobin that combines lastingly (invetsibly) with oxygen rather than temporarily. In Hb cancer, there is diminished oxygen biding and in other form of haemoglobinpathy, there is normal heme-oxygen interaction. However, with formation of a Hb that precipitates readily in hemolytic anemia, abnormal structures – Heinz bodies are in the red cells and abnormal pigment in vrine.

MOLECULAR PATHOLOGY OF HAEMOGLOBIN

Haemoglobinpathy is a molecular disease cause by familiar formation of abnormal haemoglobin. There are over 250 abnormal haemoglobins, having one form of defect in their structure or the other. These defects are pronounced when the parts of haemoglobin affected are critical to the stability of the corporate molecule or to the physiologic functions of haemoglobin. Apart from the sequence and segment affected certain amino-acid subsistent in the normal haemoglobin sequence, have peculiar physiochemical properties which are vital to normal functioning of the protein. Any form of alteration of such amino acids would eventually lead to pronounced hindering effect on the haemoglobin in the performance of its physiological function. There are certain sites which are vitally critical for the haemoglobin molecular stability and its functions such as inter chain interaction site, heme pocket, slt bridges, oxygen binding sites

etc changes to such sites produces molecular defects in haemoglobin which correspondingly result in clinically significant disease in individuals with them example are:

1. **CHANGES IN SURFACE RESIDUES**

The don't usually have serious effect except when the amino acids involved have unique physiochemical propertiers critically essentially to stability and function of haemoglobin. Example is glutamic acid (Glu0 (Relative hydrophobicity –99) which is replaced by valine (Val Relative Hydrophobicity-1.5) in the B6-position of haemoglobin molecule. This results in sickle cell disease in homozygous inheritance. In contrast in HbE lysine (Lys) relatively hydrophycity =4.2) is substituted for glutamic acids in B26 position. This produces no clinical manifestation in both homozygous and heterozygous states. This can be adduced to the fact that the difference between their relative hydrophybicities is not as high as that between Glu and Val and the preference of both amino acids for water. More so, lysine has preference for water while valme has preference for non-polar solvent.

2. **CHANGES IN INTERNALLY LOCATED RESIDUES**

These often destabilize the haemoglobin molecule. The internal environment of the molecule is hydrophobic being lined by hydrophobic amino acid residue. Thus, a change of any of these residue to hydrophillic ones produces instability. This is seen in Hb Hammersmith where phenylalamine (phe) on position B-142 is replaced by serine (ser). In Hb Bristol, valine in position B67 is replaced by Asp artic acid, (ASP) a polar group in contact with

the heme, which partially includes the heme-pocket. This weakens the binding of the heme to the protein by facilitating access of water to the subunits otherwise hydrophobic interior. Haemoglobin also destabi izes by the disruption of elements of Hs secondary, tertiary or quaternary structures. The instability of Hb Bibba result from substitution of a helix breaking proline (pro), for leucine (Leu) in A136 position. Instability of Hb savannah is caused by substitution of value (Val) for the highly conserved Glycine (Gly) in position B24, located on B-helix, where it crosses the El-helix with insufficient clearance for side chains longer than an H-atom. The A_1-B_1 contact does not significantly dissociate under physiological conditions, but may do so up on structure alteration. This occurs in Hb philly in which tyrosine (Try) position A35, which participates in the H-bonded network; that helps knit together the A_1-B_1 interface, is replaced by phenylalamine (Phe).

3. CHANGES AT THE BINDING SITES

Changes stabilizing metheamoglobin: changes at the oxygen binding site that stablize the heme in Fe^{3+} oxidation state eliminate the binding of oxygen to the defective subunits such metheamoglobins are designed as Hbm and individuals with them are said to have metheamoglobin emia. They have bluish skin, a condition known as cyanosis, which results from the presence of deocy-haemoglobin in the arterial blood. All known metheamoglobins arise from substitutions that prevents the Fe atom binding with amionic oxygen atom ligand. In Hb Boston, the substitution of tyrosine (Try) for the distal Histidine (His) in A58 position result in formation of a 5-coorsinate Fe^{3+}-compex; with

phenol ate ion of the mutant Tyrosine displacing inidazole ring of His 87 as the apical ligand. In Hb Milwaukee, the A-carboxyl group of the Glutamic acid (Glu) that replaces valine (Val) in position B67 forms an ion pair with a 5-coordinate Fe^{3+} complex. Both phenol ate and gluamate ions in these metheamoglobins so stablize Fe^{3+} oxidation state that metheamoglobin reductase is in effective in converting them to Fe^{3+} form. In HbM1 wate, Histidina (His) in position A-87 is replaced by Tyrosine (Try) Generally, metheamolgbins have Hill's constants of approximately 1.2, and low cooperatively.

HbM heterozygotes have no apparent physical disabilities which could result from defective function of their haemoglobins. However, the homxygocytes for HbM are unknown, which is blantanlty lethal.

4. CHANGES AT THE A_1-B_2 CONTACT OFTEN INTERFERE WITH HAEMOGLOBINS QUARTENARY STRUCTURAL CHANGES

Most such changes produce haemoglobins, which have increased oxygen affinity (Os) that they release less than normal amounts of oxygen in the tissues. Individuals with such defects compensate for it by increasing the concentration of haemoglobins have ruddy complexion some amino acid substitution at the A_1-B_2 interface results in an increased oxygen affinity. Individuals with such haemoglobins are cyanotic. Amino acid substitution at A_1-B_2 contact may change the relative stabilities of haemoglobins R and T forms, thereby altering its oxygen affinity. For example, the replacement of Asp artic acid (ASP) at position B99 by Histidine (His0 in Hb Yakima, eliminates the H-bond at the A_1-B_2 contact that stabilizes the T-form of haemoglobin.

The interloping imidazole ring also acts as a wedge that pushes the subunit apart and displaces them low with the R-state. This change shifts; T=R equilibrium almost entire to the R-state, which results in Hb Yakima having an increased oxygen affinity and a total lack of cooperatively. In contrast, the replacement of asparagines (ASN) in position B102 by thrcmine (Thr) in Hb Kansas eliminates the H-bond in the A_1-B_2 contact the stabilizes the R-state so that this variant remains in the T-state upon binding oxygen. Hb Kansa, therefore has a low oxygen affinity and a low cooperatively.

2.4 SICKLE CELL ANAEMIA

Sickle cell anaemia is one type of haemoglobinopathies caused by a single point substitution of hydropholic amino acid; valine for hydrophilic amino acid. Glutamic acid in position 6 of the B-chain. The point mutation in the protein sequence brings about drastic physicochemical and physiological changes resulting in the defects. A few among the physicochemical, changes of sickle cell anaemia are lower solubility decreased electrophoretic movement 2 units decrease in amionic change, altered quarternary structure etc. The mutation cause sickled cell haemoglobin (Hbs) to aggreqate into filaments of sufficient size and stiffness, which deform erythrocytes to a sickle or creasent shape. This is a remarkable example of influence of primary structure on quaternary structure.

In every cycle of their journey through the circulatory system, the red cells must squeeze through flexible capillary blood vessels which are smatter in diameter then they are. In sicker, many cells assume crescent shape under

conditions of low oxygen tension typical of capillaries. The sickling increases red cell rigidity, which hinders their free passage through the capillaries. The sickle cells thus impede the flow of blood in the capillaries such that in a sickle cell crisis, the blood flow in some areas may be completely blocked. This give rise to extensive tissue damage and excruciating pain. Also, sicklers suffer from haemolytic anaemic, condition characterized by red cell destruction. This is because the increase mechanical fragility of red cells, halves the normal 120 days life time of these red cells.

In case the sickle cells plug the capillaries of the vital organs in the body such as the brain the heart, the kidney, lungs liver. It will deprive, them of both nutrients and oxygen. The effect may be an instant death, stroke may occur in the brain or paralysis of some part of the body, heart failure and kidney failure, hepatic disfunction, respiratory paralysis and spleen infractions are common complications. The debilitating effects of this disease are such that individual with sickle cell anaemia rarely survives to maturity but modern treatments now provide life – long management and not therapy.

1. SYMPTOMS OF SICKLE CELL ANAEMIA

Sickle cell anaemia is characteriser by severe chromic anaemia. This clinical manifestation is punctuated by painful crises which are due to blockage of capillary beds in various organs with masses of red cells. This gives rise to fever, and episodic pain in the chest, abdomen or joints that are difficult to distinguish from the effects of other diseases. The blockage of capillary beds in vital organs such as the brain, Heart, Liver and kidney prevents the flow of

nutrients and oxygen to the cells of these organs. Death from anaemia from infections and ultimately from heart, kidney failures and stroke in the brain often occurs before the age of 35 – 40. Tissue death may also occur in the liver leading to ceasure or defective performance of liver metabolic function.

II. DIAGNOSIS OF HBS

Sickle cell anaemia is diagnosed by

(1) Electrophoresis

(2) Recombrant DNA technique

(3) Chemical interaction which leds to strinkage of the red cells due to oxidation

III. TRANSMISSION OF SICKLE CELL DISEASE

Sickle cell anaemia like other haemoglobinopathies and inborn errors of metabolism is a molecular disease. As such, it is transmissible to progeny by genetic inheritance from both parents, the serious and ultimate fatal disease, sickle cell anaemia, is the consequence. The cells of all higher animals but germ cells have two homologous copies of each chromosome (diploid) with the exception of sex chromosome (haploid). An organism may be heterozygous or homozygous for a gene, if it's cell respectively bears one or two copies of the gene. The haemoglobin of individuals who are homozygous for sickle cell anaemia is almost entirely sickle cell haemoglobin (Hbs). But heterozygous individuals for a sickle cell anaemia have Hb that is 40% Hbs. Such carriers of

sickle cell trait lead a normal life even though their erythrocytes have a shorter lifetime than those of normal individuals.

In term of genotype, sickle cell gene manifest itself in two ways; homozygous and heterozygous. The homozygous individuals have two copies of sickle cell gene inherited from both parents. However, the heterozygous individual has a copy inherited from either of the parents. Sickle cell gene is not sex – liked i.e. it is not bias in tern of sex in this manifestation, this means that it has equal chance of occurrence in either sex. The normal adult human haemoglobin gene, gene A, is dominant over the sickle cell gene, S – gene. Thus, a heterozygous individual for sickle gene, has a copy of normal gene and a copy of sickle – cell gene. The individual is said to be a carrier of sickle cell gene. The dominancy implies that where both the normal gene and sickle cell gene occur together as in leterozygous individuals, the normal gene, gene A_1, previous over the sickle cell gene. As such the physiological properties of normal adult haemoglobin manifest in the blood of heterozygous individuals, though he is harmed relatively title a bearing of component sickle cell gene. The (sicklers) homozygous individuals bear the full penalties of sickle cell gene.

The inheritance of sickle cell gene follows a simple Mendeli an inheritance though the gene is inherited as a nendelian recessive. The following figures illustrate the inheritance of sickle – cell gene and is manifestation of either in carriers or sicklers in offspring's.

1. Inheritance in the offspring of normal and carrier parents.

Fig 2.4.1

Normal/ A A A S Carrier (Parent Genotype) AA AS

AA AS Genotypes of the Offspring Phenotype – 50% carriers; 50% Normal.

2. Inheritance in the offsprings of normal and sicklers parents.

Fig 2.4.2

Normal A A S S Sicklers (Parent Genotype A

S A S AS AS (Genotype of the Offspring)

Phenotype: 100% Carrier,

3. Inheritance in the offsprings of normal and sicklers parents.

Fig 2.4.3

Normal A A S S Sicklers (Parent Genotype) A

S A S AS (genotype of the offspring)

Phenotype: 25% Normal, 50% Carrier, 25 % sicklers

4. Inheritance in the offsprings of a carrier and a sicklers parents, when it is crossed.

Fig 2.4.4

Carrier A S S S Sicklers (Parent Genotype AS

AS SS SS Genotype of the Offspring)

Phenotype: 25% Carrier, 50% sicklers

Heterozygous individuals (i.e. those with as genctype) or carriers may have up to 50% of their haemoglobin as Hbs whilst the other 50% is Hba. They exhibit symptoms of sickle – cell anaemia under extreme hypoxia.

V. HAEMOGLOBIN GENOTYPE IN RELATION TO SICKLE CELL ANAEMIA

Haemoglobin genotype occurs in three characteristic in relation to Sca; normal haemoglobin genotype AA, carrier haemoglobin genotype AS and sickle – cell genotype SS.

V. SEX AND INHERITANCE

Genetic inheritance of sickle cell trait (character) is not sex linked i.e. not bias to either sex. This means that both sexes are susceptible. Phenotype manifestation of the sickle cell character is also not sex linked.

VI. FEATURES

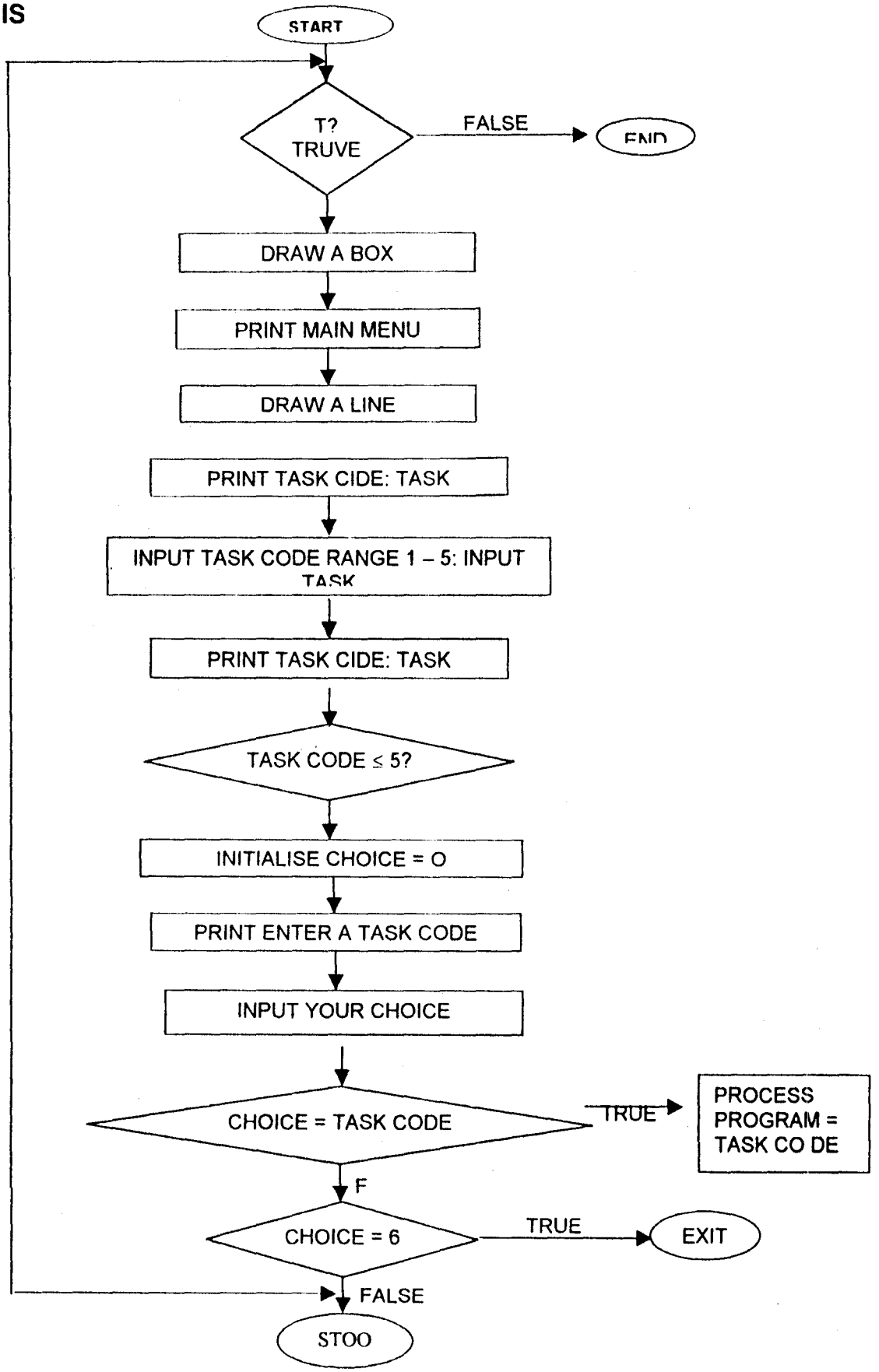
Life Span of red cells of sicklers is much shorter, ranges between 17 and 60days than a average life of normal red cells. (120 days). Sickle Cell Anaemia (SCA) has a decreased solubility in deoxygenated state (low oxygen tension). Polymerisation of deocy – HB chains to form stiff or rigid filaments which extends throughout the length of the cell (Gelation). Deformation of red cells cresent or sickle shape. Blockade of capillary beds by masses of deformed red cells. Fragility of red cells and haemolysis. HBS has low oxygen affinity T – state of HBS is stabilizer over R – state.

VII. MANAGEMENT

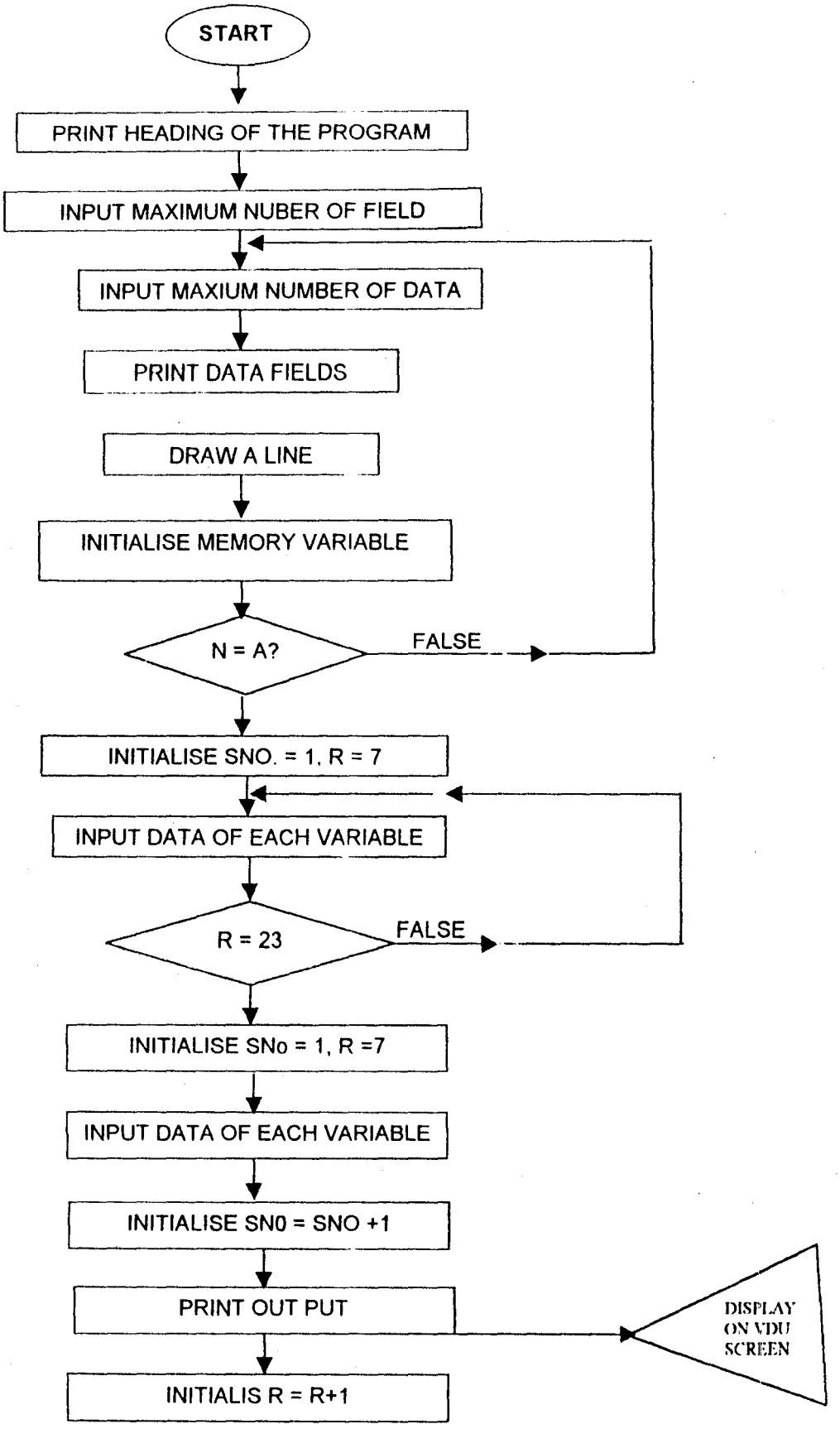
By blood transfusion, use of erythropoietin drugs like ferrous salt, vitamins and acid.

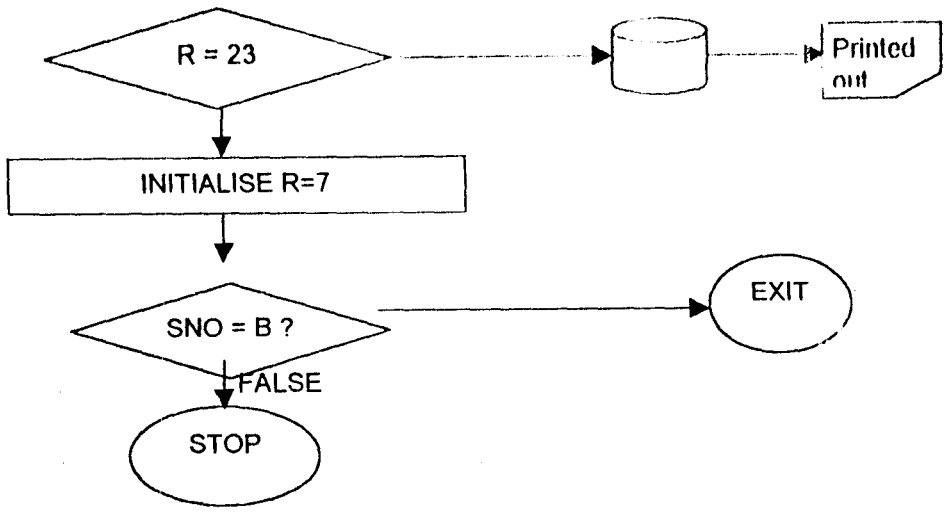
CHAPTER THREE

SOFTWARE ALGORITHMS FOR HAEMOGLOBIN/RED CELL PROTEIN ANALYSIS



REPRESENTATIVE FLOWCHARTS ALGORITHM FOR THE HUMAN HAEMOGLOBIN/RED CELL PROTEIN PROGRAMS





Application of computer in determination of Red blood protein– Haemoglobin in Human.

Analysis of a haemoglobin is the procedural study of its structure with an attempt to discover its structure.

The structure of haemoglobin the unnormal haemoglobin can be known using electro-powerful microscope. The normal health of haemoglobin have a flexible structure have dissimilar structure to the normal ones. This means haemoglobin have varied structures; a blood found unnormal haemoglobin have a sickle cell anaemia - A disease which is very fatal. It is a deadly disease.

Haemoglobin is a conjugated protein. The name of haemoglobin can be break down into two namely; Haemo, globin. Globin is the protein part of haemoglobin which consist four polypetides chain. The chains are of two types

THE TRANSMISSION ELECTRON MICROSCOPE

The Tem is used to study the details of the internal structure (ultrastructure) of cells. Extremely thin samples of the blood specimen are needed. To make these the specimen is supported in a resin blood to prevent it collapsing during cutting, and is sliced with diamond or piece with prior needle as human body to collect blood sample.

IMPORTANT FACTS

Most of the oxygen in the blood (97%) is carried in the red blood cells by a Red cell protein (haemoglobin) Hb. Haemoglobin is a respiratory pigment: it increases the amount of oxygen in the blood. The blood can carry by binding oxygen in a reversible reaction.

Oxygen does not easily dissolve in blood, but is carried in chemical combination with haemoglobin (Hb) in red blood cells.

Iron is needed to make the blood protein haemoglobin insufficient iron in the diet causes anaemia.

The very common element needed to form haemoglobin or Red cell protein (Red colouring matter) in blood is iron. Only about 0.02g of iron needed daily. Lack of iron in the diet causes anaemia (A state where there are insufficient red corpuscles in the blood).

Beans supply a good quantity of iron as do certain fruits such as figs, and tamarinds.

Life of red blood corpuscles is only 4 months. Each red blood corpuscle contain red cells protein (Haemoglobin) a red coloured chemical substance

which can combine with oxygen to form oxyhaenoglobin. Haemoglobin contain iron, hence iron is important in the diet for the arrow to make the red corpuscles. Absence of iron from the diet reduces the amount of haemoglobin in the blood, a person deficiency in haemoglobin suffers from dnaemid.

Red blood cells transport oxygen from the lungs to every cell in the body. Haemoglobin is an iron compound which readily combine with oxygen to form a bright red compound called oxyhaenoglobin.

CLINICAL CONDITIONS OF ANAEMIA

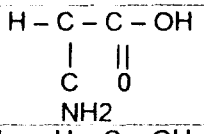
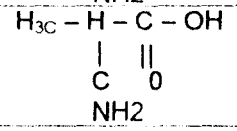
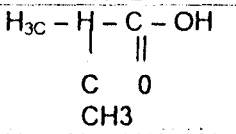
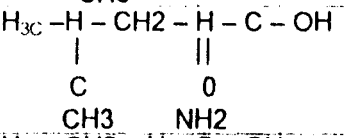
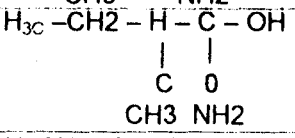
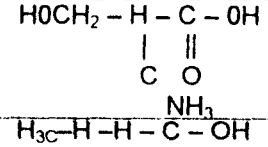
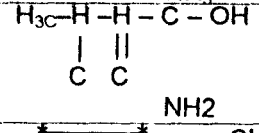
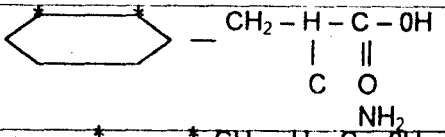
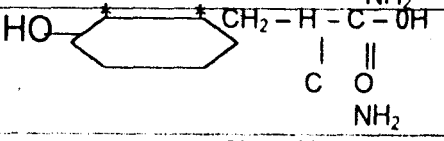
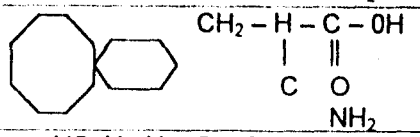
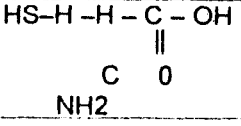
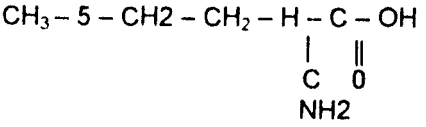
The signs are skin pallor, and in extreme cases oedema the symptoms are fatigue, lassitude headaces.

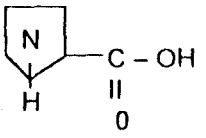
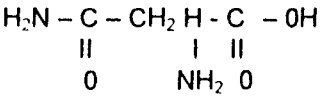
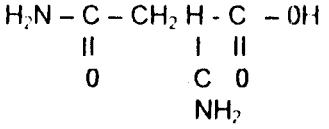
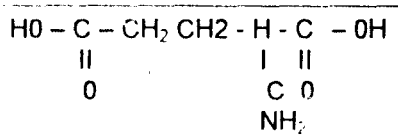
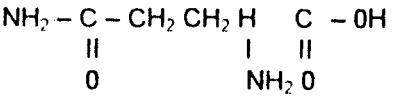
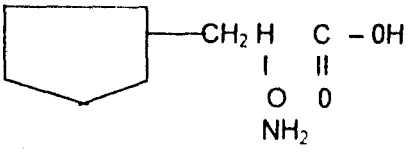
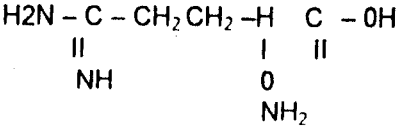
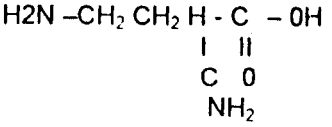
Anaemia reduces the ability to work, and the resistance of a person to disease.

CHAPTER FOUR

OUTPUTS OF THE PROGRAM TABLE 1.1.1

4.1 AMINO ACID FOUND IN PROTEIN AND THEIR STRUCTURE

	NAME	STRUCTURE
1.	Gilyine	
2.	Alanine	
3.	Valine	
4.	Leucine	
5.	Isoleucine	
6.	Serine	
7.	TH Reonine	
8.	Phenylalanine	
9.	Tyrosine	
10.	Tryptophan	
11.	Cysteine	
12	Methonine	

13.	Proline	
14.	Aspartic acid	
15.	Asparagine	
16.	Glutamic acid	
17.	Glutamine	
18.	Histine	
19.	Arginine	
20.	Lysine	

4.2 HAEMOGLOBIN PROTEIN SYSTEM EXPERIMENTATION SOME ABNORMAL HUMAN HAEMOGLOBINS RESULTING FROM POINT MUTATIONS IN THE GENE FOR A,B,C OR D CHAINS

S/No.	NAME	RESIDUE	SUBSTITUTION	MAJOR ABNORMAL PROPERTY
1.	Hb1	A16	Lys-Glu	None
2.	HbG Honolulu	A30	Glu – Glu	None
3.	Hb Torin	A47	Phe – Val	O_2 affinty ID
4.	Hb Hassharon	A47	Asp – His	Unstable
5.	Hbm Boston	A58	His – Thr	O_2 affinity
6.	Hbj Buda	A61	Lys –Asn	O_2 affinity
7.	HbA Pest	A74	Asp – Asn	None
8.	HbM Iwate	A87	His – Thr	Met Hb O_2 affinity
9.	Hb Rampd	A95	Pro – Ser	Dissociation 1
10.	Hbj Tangariki	A115	Ala – Asp	None
11.	Hb Bibba	A136	Leu – Pro	Dissociation
12.	Hb Quong size	A125	Leu – Pro	
13.	Hb Mosaka	A58	His – Tyr	Methaemolbinenia
14.	Hb Chesapeake	A92	Arg – Leu	O_2 affinity I
15.	Hbc	36	Glu – Lys	None
16.	Hbs	36	Glu – Val	Sicding, O_2 affinity I
17.	Hbj Baltimore	B16	Gly – Asp	None
18.	Hb Genova	B28	Leu – Pro	O_2 affinity I
19.	Hbe	B26	Glu – Lys	None
20.	Hb Tacoma	B30	Arg – Ser	Bohr effect I
21.	Hbm Hammer Smith	B42	Phe – Ser	Unstable O_2 affinity I
22.	Hbm Zniab	B63	His – Tyr	Unstable O_2 affinity D
23.	Hbm Sarkatoon	B63	His – Try	Meth
24.	Hbm Hyde Park	B92	His – Try	Meth b
25.	HbA Kolin	B98	Val – Met	Unstable O_2 affinity D
26.	HbD Punjab	B121	Glu – Asn	O_2 affinity I
27.	Hb Bethesda	B146	His – Arg	O_2 affinity I

28.	Hb Bethesda	B145	Tyr – His	0_2 affinity I
29.	Hb Hiroshima	B146	His – Asp	0_2 affinity I
30.	Hb Cowtown	B146	His – Asp	0_2 affinity I

4.3 SOME ABNORMAL HAEMOGLOBINS WITH DEFETED RESIDUES, EXTENDED SEQUENCES OR SEQUENCE RESULTING FROM CHAIN DUPLICATION

TYPE OF MUTATION	NAME	STRUCTURE	FUNCTIONAL
eletion	Hb leiden	B6 or B7	Abnormalntes
	Hb Tochigi	B56 – B57	Unstable 0_2 affinity I
	Hb Gum Hill	B91 – B95	Unstable
	Hb Conventry	B141 Leu – o	
		(Leu – His – Cys – Asp – Lys)-o	0_2 affinity
xtended	Hb constant	B141 Arg in A-chain is not	
	Spring	Carboxy I Terminal and chain	
		extended for 31 additional residues	
	Hb Tak	B146 is not carboxy I terminal	0_2 affinity and chain extended for 10 additional residues
	Hb Koya Dora	A141 Arg not	

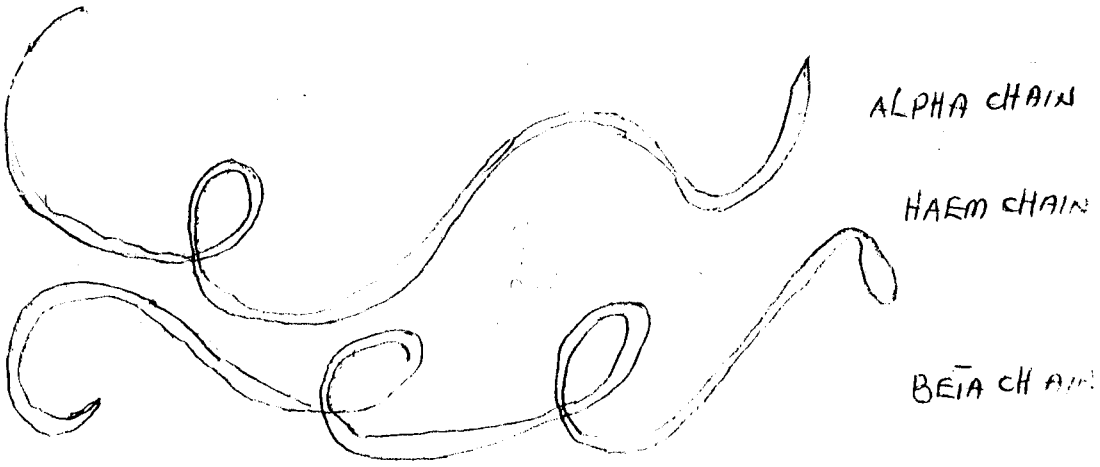
		carboxy/terminal and chain extended for 16 – 17 additional residues	
Frame Shifi	Hb wayne	A131 – 141 Frameshift In Codons To give the Sequence. Thr – Ser – Asn – Thr – Val – Lys – Law – Glu – Pro – Arg – Cooh at carboxyl terminus.	
Insertion	Hb Grady	A118 – A119, 3residuce Inserted between B118 Thr and B119 Pro	
Fusion	Hb Leprore	First third of sequence like G-chaine	

4.4 PHYSIOLOGIC ASPECTS OF SOME ABNORMAL HAEMOGLOBINS

S/No.	HAEMOGLOBIN	RED	SUBS	REGION	P50	BE	HILLS	CONC	DPG
1.	Hb Chesapeake	A92	Arg-Leu	A1 – B2	19	N	1.8	16-18	-
2.	Hb Yakima	B99	Asp-His	A1 –B2	12	N	1.1	-17	N
3	Hb Kempsey	B99	Asp-Asin	A1 – B2	10	N	1.1	-20	-
4.	Hb Rad diffe	B100	Asp-Ala	A1-B2	12	N	1.1	-20	N

called alpha and Beta. Alpha and Beta are of the same length (140 animo acids but have slightly different composition. Haem is non- protein prosthetic group which joined to globin. Haem contain an atom of iron enclosed in a ring structure.

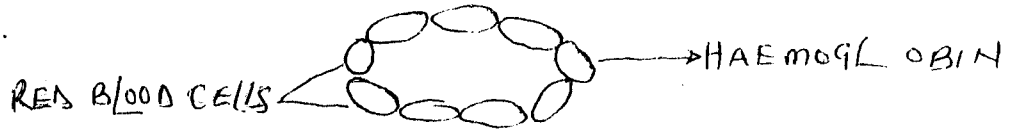
Haem group can combine with one molecule of oxygen to form a process called oxygenation. The combination of this process can not be regarded as oxidation because iron does not loose any electron and is not chemically oxidized. Each molecule of haemoglobin are able to combine with a maximum of four molecules of oxygen



STRUCTURE OF HAEMOGLOBIN

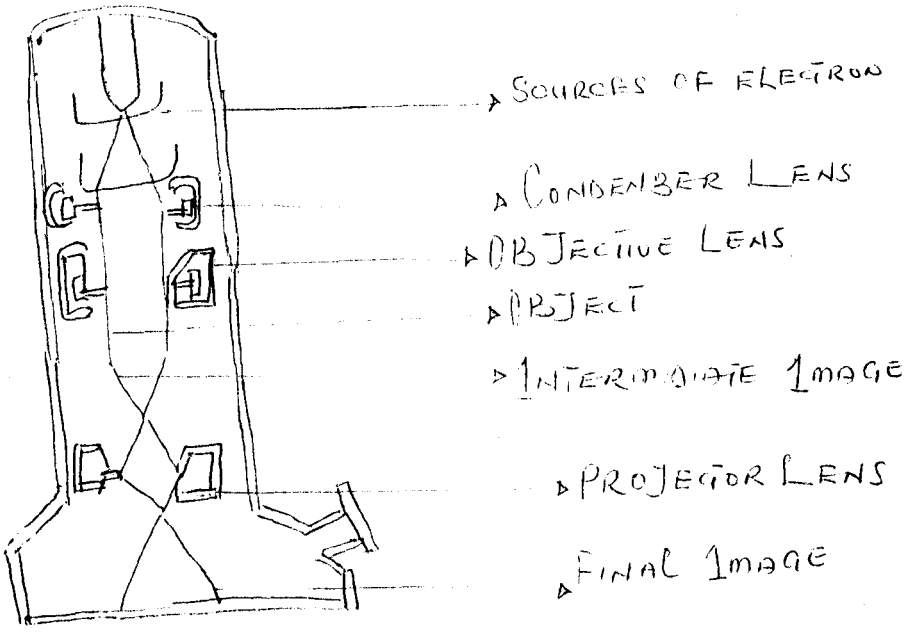
Most of the oxygen supplying mammalian cells is carried around the body by haemoglobin. The total haemoglobin content of blood is about 750g, and is normally confined within red blood cells (Eythrocytes).

Computer can be used to decide the structure of haemoglobin through scanning electron micrograph of a small blood vessel containing red blood cells.



The red blood cells or corpuscles (erythrocytes) which are round disc or bi concave and have no nucleous are filled with red protein (Haemoglobin). The human haemoglobin is confined within cells and has a relative molecular mass of about 68000.

APPEARANCE OF SCANNING ELECTRON MICROSCOPE



A transmission electron microscope. The beam of electron is emitted from the eletron gun, a heated filament, electrongnets bend of beam.

THE SCANNING ELECTRON MICROSCOPE

The SEM is use to produce three-dimensional image of the surface of specimens. Electron are reflected from the surface of a specimen stained with a heavy mental. This enable the SEM to produce image of whole specimens e.g. Red protein (haemolgobin). It is used to study dead specimen stained with heavy metal.

5.	Hb Brigham	B100	Pro-Leu	A1-B2	19.6	N	0	16-19	N
6.	Hb Demark Hill	A95	Pro-Leu	A1-B2	1	-	1.8-24	-13	-
7.	Hb san Kansas	B109	Asn-Thr	A1-B2	16.4	N	-20	-17	N
8.	Hb Kansas	B109	Tyr-Cys	$A_1 - B_2$	-70	N	-1	-14	-
9.	Hb River	B145	Tyr-cys	SALT	12.9	N	1.1	16.20	-
10.	Hb Andrew	B144	Lys-Asn	SALT					
11.	Hb Syradlse	B143	His-Pro	DPG	11	D	N	-20	N
12.	Hb Rabal	B82	Lys-Thr	DPG	18	N	N	-19	D
13.	Hb Providence	B82	Lys-Asp	DPG	D	D	2.5-2.7	-	DD
14.	Hb Hearthrow	B102	Phe-leu	Heme	I	N	-1	16.21	-

The P50 of 0_2 required for saturation of whole blood containing the abnormal haemoglobin for normal adult haemoglobin in whole blood it is 27± 2 mm Hg at PH 7.4 and physiologic temperature of 37^{oc}. Conc in Hills equations for normal value of normal whole blood is 2.8 ± 0.2, Conc in Hill equation = Normal adult value are 14 ± 2 for female and 16 ± 2 for males.

Res = Residue Subs = Substitution Region = region of molecule affected P50 = P_{50} of oxygen BE = Bohr effect, Hill = n< in Hills Equation, Conc = concentration in gl 100ml, DPG = DPG interraction, salt = salt Bridges DPG = DPG site.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

CONCLUSION

The exact description of a protein structure in term of its primary, secondary, tertiary and quaternary structures determines the specific chemical and biological properties of the protein in the native state. Similarly, genetic trait (character) are expressed through the synthesis of protein which play dynamic or structural roles that responsible for establishing the traits to be expressed. Thus the alteration in one form or the other of these structures correspondingly affect the physicochemical properties of the in concern and this in turn affects its chemical and biological functions.

In this study, which is restricted to the primary structure of the choice protein – haemoglobin it has been shown that the structural alterations in the normal sequence of amino acids of the protein (primary structure) result in a lot of functional abnormalities. These abnormal functions can be categorized into classes.

a) The life – tolerant ones (b) The life compatible (c) The fatal one (d) Those that pose constant threats to good health – disease.

Among the first class one HbD Punjab Hb Abbru330, Hb Bethesida and many others, while Hbc, Hb1, Hbc, pest HbE, Hbj tangariki and Hb Baltmore fall into the second class. The fatal structural changes in the Haemoglobin molecule are experienced in Hbm Saskatoon, Hbm hyde pack, Hbm Iwate Hbm Uwankee and other methaemoglobinemia. Those structural or functional

abnormatities resulting in disease area Hbc Harlen Hbs (sickle cell anaemia). Hb Travis and their likes. While the classification into these groups is not by any means exclusive, it can also be noted that the defective functional abnormalities ranging from life-compatibility to fatality also merge. Thus, the molecular structural changes in haemoglobin protein form the basis of molecular pathology of haemoglobinpathies.

For now, genetic disease defile any curative measure, it is hoped however that with the advancement of genetic technology, tomorrow may see the technical possibility of "gene therapy" which may serve as panacea to certain human genetic disease, not only to the sufferers but also serve to prevent such disease in their progeny. Gene therapy may also be used to mimic the human familiar disease in experimental animal so that they can be studied more carefully. For the genetic therapy to be possible there must be insertion of normal genes into human so matic cells of a defective tissue or organ to cure the patient, while insertion of normal genes into germ cells rather than into human somatic cells. Cell is necessary to prevent transmission of the disease to the progeny of the patients. This is envisaged if ethical considerations do not rule against the design of experiments intended to alter germ cell characteristic.

5.2 RECOMMENDATION

Disease generally are caused by a change in normal or health external and internal environments of an organism. The external environment includes the physical, chemical, ociological and cultural factors. All these factors as

components influence the external environments. The internal environment comprises of genetic component and is influenced by the intrinsic factors of genetic make-up. Consequently, certain it health conditions are not attributable to purely genetic dispositions of individuals involved. This lend credence to racially determined predisposition of certain diseases. Nevertheless the external environment of man remains the major threat to his health, this is evidenced in virtually all infectious and communicable diseases caused by pathogenic organisms.

Genetically determined disease often do not lend themselves to curative measures, at best they can be medically mamaged to tolerate life. Some degree of prevention can be achieve v;a health education and genetic comselling. Fortunately, familial diseases are not as widely spread as other common diseases associated with external environment. While man can do little to influence his genetic make-up, he almost entirely controls his external environments. Man does not only react with his environment, but also he is a vital factor of his own environment. Besides the phenotypic manifestations of some genetically determined diseases require homozygous inheritance of the traits rather than heterozygous inheritance. The genes coding for the traits are often recessive to the normal and dominant genes, these facts seriously reduce the number of potential candidates of such diseases.

In case where the defective gene are dominant over the normal genes, they are not at all compactable with life, except if such functions performed by the proteins are not essential and critical to life. Some homozygous inheritance

may be out-nightly fatal and not at all life-compactible e.g. homozygous of metheamoglobinemia in which the genetically determined haemoglobin has its Iron in Fe^{3+} oxidation state is unknown. Pursuant to the foregoing, some familial disease are racially inclined, while some are sex-linked i.e discriminates with regard to sex of the individual patient; the unfortunate susceptible either sex suffers from the disease.

As the familial diseases one not curable and there is virtually noting we can do to influence our individual genetic components, the logical avenues that can be exploited is in the prevention of such diseases, especially those that manifest in homozygous state. This could be achieved by health education and genetic consetting. Taking the case of sickle cell anamia as a classical example, and derived from the genetic prospects of the offspring, it is not genetically advisable for sickle – cell trait carrier pairs to contract a martial sexual relationship that would lead to birth of sickler child. For these parental genotypes the chance and percentage of sicklers in their children is 25% and the sickle –cell carriers are 25%, the remaming 50% concerns with normal children. Worse still, is the phenotype prospect of the offspring of a sickler and a carries parents (5% sicklers).

Also important is the knowledge of the environmental conditions that are health to patients of genetic diseases, or carries of such disease traits. For instance, stickler are not compatible with cold weather. (Low temperature precipitates sickling). Malaria prone areas are not conducive to health of sicklers (malaria aggravates the sickle-cell anaemia conditions, since it

produces pyrexia or fever, anaemia and malaise). Poorly ventilated area or highly populated air is detrimental to or precipitates sickling. There is also the need for regular use of erthropoetic drugs and vitamins to compensate for loss during haemolysis. Stress induces metabotic rate of the body and this leads to decrease in PH (acid-base measurement) i.e acidity increase. Low PH of high acidity weakens the binding of oxygen with the oxygen carriers protein-haemoglobin, although it at thesame time enhances oxygen with haemoglobin oxygen complex to the tissues. However, since stress requires increases oxygen delivery, which cannot be met by sickle haemoglobin Hbs in its oxygen delivery. Thus it is advisable for sicklers to avoid excessive muscular activity and stress.

Through at low temparature, the binding of haemoglobin to oxygen is higher, resulting in increased percentage of Hb saturation at constant partial pressure of oxygen; yet the oxygen delivery of Hbs (as in normal Hb) diminished at low temparature. Thus, the oxygen-delivery in hypothermic condition does not commen surate to oxygen need of the body. Mores; there is decrease in metabolics rate of the body and thus corresponding decrease in production of carbodioxide and latic acid. Since oxygen delivery increases with increase in acidity, hypothermia does not augur well physiologically, for sicklers. In the contrary, high temparature weakens Hb's oxygen affinity implying decreased saturation of Hb at constant partial pressure of oxygen in the inhale air. However, high temparature has enhancing unloading of oxygen to the respiring tissue. Consistent with this, is the fact that at high temparature,

there is increased metabolic rate requiring a rise in oxygen delivery from the oxygen haemoglobin complex yet the percentage of oxygen delivery coupled with the decreased saturation of sickle haemoglobin with oxygen in pyrexic or malaria conditions does not meet up with required amount of oxygen of the respiring tissues. Thus, pyrexia, or malaria is physiologically to the sickling conditions.

Another point worth considering is the effect of attitude on sickling. The solubilities of gases in blood are limier proportionally to their partial pressure. At high and low altitudes, there are decreased and increase solubilities respectively of these gasses in the inhaled air. Decrease oxygen affinity of Hbs coupled with decreased solubility of oxygen and other gases at high altitudes would drastically impoverish the tissues of oxygen. This condition would definitely aggravate the hypoxic of sickling and may be fatal in low altitudes; these are increased solubilities of gases in the blood, (however the low affinity of Hbs for oxygen) leading to increased percentage saturation of Hbs with oxygen. However, the oxygen delivery of haemoglobin is independent of external partial pressure of the inhaled gases, but rather depends on intrinsic property of the haemoglobin, thus sickles are not a physiological advantage low altitude. Rather on returning to the normal atmospheric pressure, sicklers like normal individuals would experience bubbling out of gases from the blood at reduced pressure. A condition reffered to as "bend" which is seriously painful.

Drugs and sickling –oxidising antimalaria and other drugs are not desirable for sicklers, since they produce oxidative haemolysis of red cells

resulting anaemia; in Glucose – 6 - phosphate dehydrogenase –deficiency individuals. Sicklers may not be necessarily deficient in this reductive enzyme (which reduce hydrogen peroxide produce from metabolic reactions, that causes lysis of red cell), but it is safer to avoid this oxidation drugs, which not only precipitates but also aggravates anaemia in sickleers. Sickle cell trait carriers do not experience the phenotypic manifestation of the defective gene, however, under extreme hypodia, the symptoms of sickle cell anemia may set in. This is due to inherit component of the defective gene in haemoglobin genotype of carriers.

Finally, there is a need for individuals to known his or her haemoglobin genotype, awareness of this and of the possible consequence of careless and unguided genetic disposition to the progeny as it is in sicklers; would go a long way to reduce, if not totally prevent disease arising from genetic doom.

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APPENDIX I GLOSSARY OF TERMS

Phenotype	The observable trait(s) that result from the genotypes in cooperation with the environment
P50	Partial pressure corresponding to 50% saturation
WBc	White blood cell or leucocyte
SDc	sodium dodecylsulphate (petergent)
TLc	Thin layer chromatography
PO_2	Partial pressure of oxygen
PKn	Negative logarithm of the lonination constant of a acid
RBC	Red blood corpuscles
PH	Acid base measurement
0_2	Oxygen molecule
NMR	Nuclear magnetic resonance
Mb	Myoglobin respiratory transport protein in the mush
Kep	Equilibrium constant for a reversible reaction
HBs	sickle cell haemoglobin
Isoelectric point	The PH at which a protein has no net change
HHB	Deoxygenated haemoglobin or deoxy- haemoglobin
Hb	Haemoglobin respiratory transport protein in the blow
Haploid	A cell containing only one chromosome of each type
Genotype	The genetic characteristics of an organism distinguished from its observable characteristic or phenotype.
Genome	The total genetic content of a cell

GABA	Gamma amino butytricacid, a chemical neurotransmitter
G.6. PD	Glucose – 6 – phosphate dehudrogenase
Fe2+	Iron atom in 2 – oxidation state
Eleltophoresin	is a separation technique for electrically charqed substances the principle of operation is based on the netchange on the substance
Haem	is a non protein prosthetic groups which joined to Globin. Haem contain an atom of iron enclosed in ring sructure.
Diploid	a cell that contains 2 chromosome 2N of each type.
Globulm	molecule transporting protein animal
Haemoglobin	is a conjugated protein oxygen carrying substances in the red blood cells of vertebrate.

APPENDIX II HUMAN HAEMOGL[illegible]N SOFTWARE PROGRAM

```
AN HAEMOGLOBIN PROTEIN PROGRAM
000, 200


: Print: SLEEP 2
6, 23: Color 13, 6: Print "ENTER CURRENT PASSWORD": LOCATE 5, 39: Color 7: Print
7, 38: COLOR 10: INPUT PW$


'BIN" Or PW$ = "bin" Then GoTo 9 Else GoTo 310
 20: Print "YOU ARE NOT AN AUTHORISED USER": GoTo 400
OCATE 10, 25: Color 10, 6: Print "YOU ARE WELCOME TO HUMAN HAEMOGLOBIN
 PROGRAM DESIGN"
 13, 30: Print "PLEASE WAIT WHILE PROGRAM LOAD": SLEEP 5: GoTo 4


oTo 14
SLEEP 4

 3, 13: Color 11: Print "BY OLANAMU JACOB KEHINDE": SLEEP 5
 5, 13: Color 10: Print "(P.G.D IN COMPUTER SCIENCE)": SLEEP 5
 7, 13: Color 16: Print "PGD/MSC/2003/2004/1129": SLEEP 5
 9, 13: Color 15: Print "IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE
 OF POST GRADUATE DIPLOMA IN COMPUTER SCIENCE OF THE FEDERAL
RSITY OF TECHNOLOGY, MINNA": SLEEP 5
 13, 13: Color 18: Print "NOVEMBER, 2004.": SLEEP 5
 15, 13: Color 11: Print "APPLICATION OF COMPUTER IN THE DETERMINATION OF
RY STRUCTURE OF HUMAN RED CELL PROTEIN-HAEMOGLOBIN": SLEEP 5
 17, 13: Color 11: Print "THE STUDY CONCERNS WITH STRUCTURE-FUNCTION
IONS IN THE HAEMOGLOBIN": SLEEP 5
 19, 13: Color 11: Print "GLOBIN MOLECULE AND POINTS OUT VARIOUS
RMALITIES": SLEEP 5
 20, 13: Color 11: Print "AND THEIR CONSEQUENCES ARISING FROM CHANGES IN
RY STRUCTURE": SLEEP 5
 21, 13: Color 11: Print "(SEQUENCE OF AMINO ACIDS) OF THE TRANSPORT
IN": SLEEP 5

S: SLEEP 4
 4 To 18
12, 7
TE 4, 18: Print String$(32, "*")
TE I, 18: Print "\"
TE I, 50: Print "\"
I
LS: SLEEP 4
```

```
EEP 1
 3
nt: Print: SLEEP 2

S IS THE MODULE THAT PROCESSES  SOME ABNORMAL HUMAN HAEMOGLOBIN
ING FROM PINT
TATION IN THE GENES FOR AIBID OR 9-CHAINS
 1, 10: Colo 1: Print "SOME ABNORMAL HUMAN HAEMOGLOBIN RESULTING FROM

 2, 15: COLOR 1: INPUT "S/NO"; SNO$
 3, 15: COLOR 1: INPUT "HAEMOGLOBIN"; HEAGLO$
 4, 15: COLOR 1: INPUT "RESIDUE"; RES$
 5, 15: COLOR 1: INPUT "SUBSTITUTION"; SUBS$
 6, 15: COLOR 1: INPUT "REGION IN MOLECULE AFFECTED"; REMOAFF$
 7, 15: COLOR 1: INPUT "BOHR"; BEOFF$
 8, 15: COLOR 1: INPUT "N = EQUATION"; NEQU$
    COLOR 1: INPUT "CONC (E) g/100ml"; CONCgml$
    COLOR 1: INPUT "DPG"; DPG$
 11, 15: COLOR 1: INPUT "COMMENT"; COMM$
 1, 15: Color 9: Print "MUTATION IN THE GENES FOR AIBID OR 9-CHAINS"



4
 10: Print "THIS IS THE PRIMARY STRUCTURAL RESULT"
 LOCATE 5, 2: Print "SEGEMENT THAT TEST SOME ABNORMAL HAEMOGLOBINS"
E 13, 2: Print "I": LOCATE 10, 5: Print "GLYCINE":
E 10, 16: Print "GLY": LOCATE 10, 44: Print "5.97"
E 16, 53: Print "o": LOCATE 13, 63: Print "H---C--C---OH":
E 19, 69: Print "O": LOCATE 15, 68: Print "2"
E 22, 5: Print String$(80, 196)
= "bm 536, 224 cl5, e2,r2,f2,g4,r4"

4
4
E 24, 2: Print "2": LOCATE 18, 5: Print "ALANINE":
E 18, 16: Print "Ala": LOCATE 18, 23: Print "2.3": LOCATE 18, 29: Print "9.9"
E 24, 37: Print "___": LOCATE 18, 44: Print "6.0"
E 24, 53: Print "0.5": LOCATE 20, 63: Print "H---C--C--C---oH":
E 25, 68: Print " / ": LOCATE 18, 68: Print "; I  "; ""
E 25, 68: Print "/": LOCATE 22, 68: Print "NH"
E 25, 72: Print "//": LOCATE 22, 72: Print "O"
= "bm555, 350, cl5, e2, r2, f2, g4, r4"
= "bm503, 314, cl5, e22, r2, f2, g4, r4, d3, g2, i3"
```

```
CLS
SLEEP 4
LOCATE 10, 2: Print "3": LOCATE 10, 5: Print "VALINE":
LOCATE 10, 16: Print "Val": LOCATE 10, 23: Print "2.3":
LOCATE 10, 29: Print "9.7": LOCATE 10, 37: Print "___": LOCATE 10, 44: Print "5.96"
LOCATE 10, 53: Print "1.5": LOCATE 12, 61: Print "HC---C-C--CH--C-C--OH"
LOCATE 11, 66: Print "/": LOCATE 10, 66: Print "H"
LOCATE 13, 66: Print "/": LOCATE 13, 70: Print "/":
LOCATE 14, 65: Print "CH": LOCATE 14, 69: Print "NH"
LOCATE 13, 73: Print "/": LOCATE 14, 73: Print "O"
LOCATE 16, 5: Print String$(75, 196)
draw$ = "bm488,185,cl5,e2,r2,f2,g4,r4,d3,g2,l3"
draw$ = "bm530,218,cl5;e2,r2,f2,g4,r4,d3,g2,l3"
draw$ = "bm560,218,cl5,e2,r2,f2,g4,r4"

SLEEP 4
LOCATE 18, 2: Print "4": LOCATE 18, 5: Print "LEUCINE"
LOCATE 18, 16: Print "LEU": LOCATE 18, 23: Print "2.3"
LOCATE 18, 29: Print "9.7": LOCATE 18, 37: Print "___": LOCATE 18, 44: Print "5.98"
LOCATE 18, 53: Print "/": LOCATE 20, 62: Print "HC-CC-CH-C-C-OH"
LOCATE 18, 37: Print "___": LOCATE 18, 44: Print "5.98"
LOCATE 18, 53: Print "1.8": LOCATE 20, 62: Print "HC-C-CH-C-C-OH"
LOCATE 19, 66: Print "/": LOCATE 18, 66: Print "H"
SLEEP 4

CLS

400 End
```