TITLE PAGE

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

BY

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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 PROJECTS IN THE DEPARTMENT OF MATHEMATICS/COMPUTER SCIENCE, SCHOOL OF SCIENCE, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA.

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DEDICATION

To God belongs all glory, for in all changing fortunes of life, His mercy endureth forever (PS. 136). This project work is dedicated to my parents Late Daniel Ishola Oyekale and Mama Dorcas A.F. Oyekale whose stands and courage during various twists of fortunes of life serve as enduring confidence and challenge to always forge our way forward in the faces of threatening impossible circumstances, their collective will to make us first in the commity of equals and all-round excellent in our various pursuits serve as sources of untiring inspirations and lasting happiness to us all-children.

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ABSTRACT

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

Thus, any deficiency of the components of these systems would lead to altered 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 protein. This situation is evidenced in various molecular abnormalities of haemoglobin stucture, which thus result in haemonglobinopathesis of which sickle cell anaemia is one.

A studious look into the molecular structure of heamoglobin in relation to its functions forms the bases of structure function analysis which is an eye-opener to various familiar diseases. In this study, various charges in structure of haemoglobin with their correlating pathological consequeces are elucidated as genetic molecular diseases. Genetic diseases defile the solution of therapy, consequent upon this, is the genetic education and counselling given not only to relieve the symptous of the inborn haemoglobin-related diseases, but also aims to reduce it, if not totally eliminate the occurences of genetic diseases and victim of such, resulting from genetic doom.

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1.1

CHAPTER ONE

INTRODUCTION

Proteins form one of the classes of biologic macromolecules, involved at the centre of actions of bioprocesses. They have the most well defined physicochemical properties and consequently are generally easier to isolate and characterise than other biomacromolecules such as Nucleic acids, polysaccarides and Lipids. Protein is important in the body building in terms of cell growth, replacement of dead cells and repair of damaged cells. So important is protein, in fact, that cells are made up largely of protein as the functioning u it. In this trend, proteins perform two varieties of functions: Dnynamic and Structural. Dynamic functions of proteins include Transport, Metabolic control , Contraction, Catalysis of Chemical transformation. These can be summarily enumerated thus: Control and retgulatory proteins in translation and transcription. Histones associated with DNA, Receptor and enhancer rpoteins which control genetic expression. Ribosomal protein in translation process. Roles in contractile mechanisms - Myosin and Actin that function in muscle contraction. Transport proteins - Heamoglobin in blood, Myoglobin in muscle which transport respiratory gases. Transferin oncerns with transport of Fe^{2+} etc. Hormones such as Insulin, Thyrotropin, Somatotropin, Luteinishing hormones, follicle stimulating hormone. Peptide hormones such as Adrenocorticotropin, Antidiuretic hormone, Glucagon, Calcitonin. Thyrotropin releasing factor, Methionine enkelphalin. (Copiate-like peptide in the brain which inhibits sense of pain). Little gastrin-hormone which stimulates parietal cells to secrete acid. Vasodilator peptide-plasma brady kinni. Substance P-which serves as chemoneurotransmitter. Protective proteins - immunoglobulins and interferons which act against bacterial and viral infection respectively. Fibrin stops loss of blood on injury to the vascular system. Structural functions - formation of matrix of bone and fragments and provision of structural strenghts and elasticity to the organism and the vascular system e.g alpha-keratin has essential structural role in epidermal tissue.

Generally, protein peform the following functions:

(i) They function as enzymes that catalyse the complex set of chemical reactions that are collec tively referred to as life.

(ii) Serve as regulators of these reactions both directly as components of enzymes and indirectly in the form of chemical messengers such as hormones and receptors of hormones.

(iii) Transport and store biologically important substance eg. Metal ions, oxygen, glucose, lipids other molecules.

(iv) In form of muscle fibres and other contractile assemblies, proteins gernerate coordinated mechanical motion of numerous bio-responses including separation of chromosomes during mitosis and eye movements (Rhodopsin acquires sensory information that is processed through the action of nerve cell proteins).

(v) Immunoglobulins-proteins of immune system form an essential biodefence system in higher animals.

(vi) Proteins are the active elements and product of the expression of genetic information, the Nucleis acids are for the most part information banks upon which proteins act.

(vii) Passive but structural role - collagen which produces bones, tendons and ligaments with their characteristic tensile strenght such structures such as hairs, nails are made up of essentially proteins. As such, proteins are the building blocks of life.

Proteins are linear polimers of amino acids, which are integral subunits of polypeptides and proteins. Many amino acids and their derivatives are of biochemical importance, these alternative specialised role of amino acids, besides their roles in proteins, are examples of biologic opportunism. Such biologically important functions are:

(a) Amino acids and derivatives often functions as chemical messangers in the communication between cells e.g. Glycine, GABA , Dopamine are neurotrasmitters (substance released by nerve cells to alter their neighbours).

(b) Histamine serves as a local mediator of allergic reactions.

(c) Thyroxine - an iodine containing thyroid hormonr that generally stimulates vertebrates me tabolism.

(d) Amino acids are intermediates in various metabolic processes citruline and ornithine are intermediates in urea biosynthesis. Homocysteine - is intermediate in amino acid metabolism.

(e) S-adenosyl methionine serves as a biologic methylating agent.

(f) About 250 different amino acids have been found in plants and fungi, most of them being toxic suggests that they have protective function. Indeed, some of them are medically useful antibiotics e.g. Azaserine, B-cyano alanine etc, L-aminoacids are present in proteins but D-Amino acids are present in many organisms. They serve as constituents of bacterial cell walls, where they serve a deffensive function. D-Amino acids render the bacterial walls less susceptible to attack by the peptideses that D-Amino acids also occur as component of many antibiotics including Valiomycin, Actinomycin S, Gramicidm S. Thus, amino acids and their derivatives also have independent biologic roles as neuro transmitters, metabolic intermediates and poisons.

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

AMINO ACIDS FOUND IN PROTEIN

	NAME	A-Name	PK_A	PK_B	PK_R	I_P	RH
1.	GLYCINE	Gly	2.3	9.8	-	5.97	0
2.	ALANINE	Ala	2.3	9.9	-	6.00	0.5
3.	VALINE	Val	2.3	9.7	-	5.96	1.5
4.	LEUCINE	Leu	2.3	9.7	-	5.98	1.8
5.	ISOLEUCINE	Iso	2.3	9.7	-	6.02	2.5
6.	SERINE	Ser	2.2	9.4	13	5.68	0.3
7.	THREONINE	Thr	2.1	9.1	13	5.60	0.4
8.	PHENYLALANINE	Phe	2.6	9.2	-	5.48	0.4
9.	TYROSINE	Tyr	2.2	9.1	10.1	5.66	2.3
10.	TRYPTOPHAN	Try	2.4	9.4	-	5.89	3.4
11.	CYSTEINE	Cys	1.8	10.8	8.3	5.07	2.8
12.	METHIONINE	Met	2.2	9.3	-	5.74	1.3
13.	PROLINE	Pro	1.9	10.6	-	6.30	3.3
14.	ASPATRIC ACID	Asp	2.0	10.0	3.9	2.77	-7.4
15.	ASPARAGINE	Asn	2.0	8.8	-	5.41	-0.2

16.	GLUTAMIC ACID	Glu	2.2	9.9	4.3	3.25	-9.9
17.	GLUTAMINE	Gln	2.2	9.1	-	5.65	-0.3
18.	HISTIDINE	His	1.8	9.1	6.0	7.59	0.5
19.	ARGININE	Arg	1.8	9.0	12.5	10.76	-11.2
20.	LYSINE	Lys	2.2	9.2	10.8	9.74	-4.2

A - NAME = ABBREVIATED NAME

PKA = PK of alpha carboxylic (COOH) group.

PKB = PK of alpha amino (NH_2) group.

PKR = PK of alkyl (R) substituent

IP = Isoelectric point

RH = Relative hydrophobicity in Kcal/Mole.

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 preffence water and positive values indicate a preffence for non-polar solvent .The data with astericks are calculated values .Amino acids with double astericks are aromatic.

The specific chemical and biological properties of an individual protein should find explanation in term of an exart description of its structure in the native state . Some deficiencies of protein inpathological conditions could be traced to altered srtucture of such proteins. For example ,Sickle cell aneamia arises from the altered structure in the position 6 of B-chain of heamoglobin. The mere point substitution of water solube amino acid (Glutamic acid) by lipophillic amino acid (Valine) in the B6-position of heamoglobin chain results in deadly sickle cell disease. This single intramolecular change so alters the properties of the hemoglobin molecule that anaemia and other deffects are produced.

The functions of proteins in biologic system to maintain life are myriad, mediating several biologic life depend. When there is an equilibrium shift of the intricate normal balance either in function or quantity, there 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 threats to life - disease. The defects (Physiologic or biochemical) produced by abnormal or subnormal protein may be due to alteration in the structure of the protein. This is especially if such structural variant ocur at the active site of the protein or at the active site dependent portion ususally reffered to as allosteric site. A

curious study of the effects of altered structure of such proten in relation to its function would lead to knowledge of the structure function relationships of the protein molecule.

For heamoglobin, other equally clinically important and less severe clinical defects commonly reffered to as heamoglobino pathies do exist and arise from alteration of the primary structure of Haemoglobin by substituting of the compostie amino acids at one position or the other with other amino acids.

1.2 OBJECTIVES OF THE STUDY

The occurence of sickled cell anaemia is almost exclusive to Africans, about 7-10% of whom in the USA carry the sickle cell trait. The actual disease-sickle cell anaemia-is less common 0.3% - 2.5%. This simple statistics shows that the disease is peculiar to Africans, thus proper awareness about and tangible knowledge of the disease is necessary with a view to reducing the genetic trnsmission of the disease. Similarly, the clinical fact that the disease is incurable and fatal, coupled with only the clinical possibility of treating the clinical symptoms rather that the disease proper, evoke a social need to guide against the chance of giving rise to sicklers who will spend theri short- span lives in the menace of utter and 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 enviornments, many a sickler has been witnessed to lived out his full life span. Other important point is that sickling is neither the fault of the mother , the father, nor the child, however it is an accumulation of genetic rrors from both parents, which gives rise to the disease intheir child. Thus, there is a ned for genotype-check up to ensure marital compatibility prior to wedlock; in order to avoid the unfortunate incident.

1.3 THE SCOPE OF THE STUDY

Hemoglobinopathies 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 involve the leukocytes or white blood cells, and the platelets and the tissues in which they are formed-the bone marrow, the lymph nodes, the spleen which are often reffered to as heamatopoietic system.

The study is restricted to blood disease that involves the red cells of the blood, more specifically concerning the molecular diseases arising due to change in the structure of the transport protein

- hemoglobi of the red blood cell and how this change in structure affects the transport fnction of oxygen ie the physiologic effect and the ultimate clinical consequence.

1.4 THE TISSUE FLUID - BLOOD

Blood is a cell-containing fluid that transports oxygen, water, carbondioxide, products of metabolism and internal secretions e.g. Hormones. The blood and lymph are the important fluids connecting the diverse anatomical structures of the mammaliam organisms. Blood being a tissue that constanly cirulate throughout animals, serves as a means by which constancy of the internal environment is maintained. It is also 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 in veins following a complex but completely closed circular path. The red colour of the blood is imparted by the porplyrin ring of hemoglobin-an oxygen carying protein in red cells. The specific gravity of human blood ranges between 1.0 55 and 1.065, its viscousity is approximately 5 to 6 times that of water. The total volume of blood in vascular system approximates 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 cells per ml are present while in adult woman , the red cell count is less , about 4.8 million red cells per ml . In in fants,red cell count is more at neonatal stage than that of adult woman , but as the baby grows , the number decreases 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

1. THE PLASMA

The liquid part of the blood is the nearly colourless plasma in which are dissolved various solute constituents. The various plasma solutes are: Proteins 7%, Inorganic salts approximate 0.9%, remainders are diverse organic compounds other than protein.

1. 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 0.2-1.4. Creatine 0.2--0.9 Creatinine 1-2 Uric acid 2-6.

2. CARBOHYDRATE:- Macromolecules such as Glycogen 5-6 Polysacarides as hexoses 70-1-5 Glucosamine 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 02-1.0, 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 range between 285-675 mg/dl within the plasma. The major ones are cholesterol 130-260, Neutral fat 80-240, Esters 90-190. Total falty acids 150-500 and others.

5. The plasma proteins ranges in concentration normally in normal human adults from 5.7 to 8.0 g/dl. The proteins which can be fractionated by electrophoresis, have major fractions as

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

(ii) Alpha I-globulins 6-9% - nvolved as proteins in coagulation of blood and as protease inhibitors.

(iii) Betha II-globulin 8-9%, BI-globulins 13-14% - As lipoproteins (iv) Beth II - Microglobulins - associated with HL-A histocompatibility Antigen complex. which is implicated in Graft rejection, Autoimmnune reactions etc. (v) Cryoglobulins - Implicated during inflammatory diseases of rheumatoid arthritis, multiple myeloma etc. (vi) Fibrinogen 2.5-5.0% - chiefly responsible for the formation of blood clot during heamostasis.

The plasma solutes being soluble materials, apart from the specialised physiologic roles they play, function in osmotic regulation of the blood. Their osmoactive nature helps in maintaining normal water b alance between the blood and the interstitial fluid. The excess water and poisonous bye-products of metabolism e.g. urea, uric acid etc are passed to the kidneys for excretion. Also, some of the blood plasma solutes are used as detoxifying and conjugating agents for various endogenously produced poisons 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 utomost 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 physiologic importance are the roles played by blood plasma

solutes in the maintaince of blood viscousity and density within the normal range. These are vital to the maintaince of normal and sufficient systolic pressure which allows for blood circulation round the body. The body defence mechanism through the agents of antibodies (Immunoglobulins) is upheld by globin serving as precursor in the biosynthesis of anti bodies.

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 and plateletes or Thrombocytes.

A THE WHITE BLOOD CELLS OR LEUOCYTES:- They are the largest cells among the cellular components of blood, they are nucleated and make up to 4500 - 11000 cells per ml of blood (white cell count) in normal ranges. White blood cells comprise of three different cells: Granulocytes, lymphocytes and monocytes. Fluctuations occur in white cell count during the day, 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 ganules. They may be present for few hours in circulation and then distributed throughout tissues 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 acidic dyes.

i. Neutrophills make 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 bacterial and other fine particles and they may destroy living microorganisms. Granules of neutrophills contain potent enzymes capable of digesting many types of cell materials.

ii. Eosinophills 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 oxidations in which there are antigenantibody interactions.

iii. Basophills make up to0-40 cells per ml of blood or up to 1% of white cell count.

2. 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 cell. They are found in large numbers in bones, spleen, thymus, tensils

and lymphod tissues of gastrointestinal tract, 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 proteins and other antigens not necessarily derived from living cells.

There are two classes of lymphocytes: Class 1 contains cells which produce immunoglobulins including various tyupes of antibodies in response to stimulation by an antigen. Class II contains cells which are concerned with cell-mediated immunity. These lymphocytes participate in the rejection of transplanted tissues 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 accomlished by cell-mediated immunity. Lymphocytes migrate to the area of the graft and cause its destruction. Cell-mediated immunity is also involved inother reactions including the tuberculin reaction.

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

3. MONOCYTES: Are 300-700 cells per ml of blood or 4-8% of white cell count. They are characterised by lobulated nucleus with rounding projections. They are largest of all the white blood cells, their cytoplasms contain fine granules. they are actively phagocytic, actings as scavengers and they are found in inflammed tissues earlier than granulocytes. They are found at sites of chronic infections where they are involved in ingestion of infectious agents as well as red cells and other large particles. Monocytes are also precursors of the large phagocytic cells of the tissues called the macrophages.

THE PLATELETS OR THROMBOCYTES

They are anucleated and incapable of cell division, they adhere to each other but not tocells or white blood cells. The normal count ranges between 150,000 and 300,000 cells per ml blood. Theirfunctions is related to heamostasis, which is the prevention and control of bleeding. If thrombocyte are not present, heamostasis 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 substances essential for the

normal coagulation of the blood and they cause strinking 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 NORAMALITY
1. VOLUME MEASUREMENT	7-9% BODY WT	BLOOD GLUCOSE	80-120
2. PH (ACID -BASE MEASUREMENT	7.35 - 7.45	SERUM PROTEIN (TOTAL PLASMA)	5.9 - 7.5g
3. RED BLOOD CELL	4.5 - 5.5 $X10^6/mm^3$	ALBUMIN/GLOBULIN	1.3:1 - 2.9:100
4. WHITE BLOOD CELL	5000 - 10,000/ml	PLASMA FIBRINOGEN	290 - 500
5. PLATELETS	200,000-400,000/ml	BLOOD NON-PROTEIN NITROTGEN (NPN)	24 - 40
6. HB CONTENT	14 - 16g/100ml	BLOOD UREA NITROGEN (BUN)	8.0 - 2.0
7. HEAMOCRIT	47 - 50%	BLOOD URIC ACID	3.5 - 5
8. COLOUR INDEX	0.9 - 1.1	SERUM TOTAL CHOLESTEROL	130-250
9. VOLUME INDEX	0.9 -1.1	BLOOD PROTEIN - BOUND IODINE	4.0 -8.5g
10. BLEEDING TIME	1 -31 MINUTES	SERUM SODIUM	312 - 342
11. COAGULATION TIME	5.5 -12.5 MINUTES	SERUM POTASSIUM	14 - 21
12. SPECIFIC GRAVITY 25°C	1.05 - 1.06	SERUM CALCIUM	85 - 11.5
13. RELATIVE VISCOUSITY (38°C)	4.7	SERUM INORGANIC PHOSPORUS	2.4 - 4.0
14. SEDIMENTATION RATE	0 - 20mm (FIRST HOUR)		
15. PROTHROMBIN TIME	10 - 15 SEC.		

Values except where indicated are in milligrams per 100 millilitres. Heamocrit values:42-54% of total value in men ,37-47% of total value in women.

Volume of blood in an average person amounts to about 70ml (2.3 Oz) for each Kg (2.2Ib) of body weight. Viscousity compared to that of water it has been formed, platelets are formed in the bone marrow, but the spleen serves as their reserve store.

C. THE ERYTHROCYTES OR RED BLOOD CELLS

The human red cell s arenon -nucleated, biconcave disc with a diameter of 6-9 nm. Red cells play transport and regulatory functions. The circulating red cells and the total mass of erythropoietic cells from which they are derived are termed the Erythron.

The erythron , though dispersed organ has prime functions of transporting of oxygen and carbondioxide and maintainance of the PH of blood . The matured cells of the erythron the circulating red cells have known lifetime, in circulation about 120 days . During its life time , a red cell travels about 175 miles through the circulation .

Erythrocyte is a highly specialised cell , its cytoplasm contains 34% solution of heamoglobin, 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 mammals have most efficient red cells at least so far as oxygen carrying ability is concerned. Red cells are thin in the centre, which perhaps.increase 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 into cones,clubs dumbells as they pass through extremely narrow blood capillaries. Erythrocyte behave as osmometer, swelling and strinking with increase and decrease in osmotic pressure of the medium.Redcell contains K^{+}, Na^{+}, Ca^{2+}, Mg^{2+} as intracellular cations and the anions are Cl^{-}, HBO_3^{-} ,HB, Inorganic PO_4^{2-},2,3--diphospho glycerate. As red cells age and are removed from circulation by the cells of reticulo-endothelia system, thier hemoglobin HB is degraded. The globin of HB is hydrolysed to their Amino acid constituents, which are reutilized for protein synthesis, iron is transported by transfering to bone marrow and other tissues and reutilized for heme synthesis. The protoporphyrin from heme is degraded in reticulo-endothetialcells and the Liver, and the resulting bile pigments are excreted via bile into intestinal lumen.

Derangement of any of these processes at any phases of the life cycle of the redcell can lead to severe human disorde. The altered rates of RBC production and destruction as a result of abnormal conditions ranging from malnutrition to hereditary defects may shortenthe lifespan of the red cells, giving rise to anaemia and defective function of the erythron.

CHAPTER TWO
HEAMOGLOBIN FUNCTION

2.1 THE RED CELL PROTEIN - HEAMOGLOBIN

Human heamoglobin is a conjugate protein consisting of prosthetic groups (ie non-amino acid moietys) of hemes and four polypeptide chains which may be A,B,G or D. Fetal heamoglobin contains 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 A1, A2 chains. The same thing is applicable to B-chains, being reffered to as B1, B2, chains. Each peptide chain is conjugated to a heme group-Ferropro-top orphyrin IX. The four polypeptide chains in heamoglobin mesh together within the little space in the interior of red cell. The forces linking the four chains involve only secondary forces such as Hydrogen boundings, 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 Histidine (HIS) at 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 thus allows for reversible binding of oxygen. Also it places the heme in the invironment of low dielectric constant/ Similarly, imidazole group of His at postion 92 in the b-chain binds proximally to the heme Fe. In A -chain, His at postition 87 is proximally bound to the heme Fe. The two propionate side chains of each heme lie in juxtaposition to the positively charged Nitrogens of a Lysine and Arginine redidues at positions 82 and 104 of the B-chains. Nitrogen element of imidazole forms a coordinate bond with Fe^{2+} by donation of lone pair elections of Nitrogen,to complete the hexavalency of Fe; Oxygen binds reversibly (association) with the Fe^{2+}

In B-chain, there are 146 amino acid components. The possible number 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 possible 20^{146} B-Polypeptide chains. However, the number is tremendously reduced to few by the predetermination of the polypeptide chan sequence by

the specific gene, coding for the chain. In the polypeptide chain, if the number of chiral centres is N (all biologic amino acids are assymentric or chiral except Glycine), then 2N different possible stereoimomers and 2N-1 enantiomeric pairs of polypeptide chais that can be produced. The colour of the blood arises from the characteristic absorption spectra of the heme group (Ferriprotoporphyrin ix) 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 fully compatible with normal physiologic functions of hemoglobin that maintain life.

2.2 FUNCTION OF HEAMOGLOBIN

A. TRANSPORT FUNCTION OF HEAMOGLOBIN

In the lungs, the heamoglobin chains bind to oxygen molecules, the binding of oxygen molecule to one chain facilitates the binding to other chains in a red cell. At the same time, DPG binds to each chain or heamoglobin at the allosteric site. However, binding of 2,3-DPG to oxy-Hb is much weaker than that of Deoxy-Hb, this stabilizes deoxy-Hb over oxy-Hb. 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 saturates Hb. The physiologic effect of DPG is upon the release of oxygen in the extrapulmonary tissues where oxygen tensions are low. High DPG signifies the effeciency of oxygen delivery in the blood. Rise of DPG is noted in red cells in conditions associated with tissue hypoxia - eg Anaemia, Cardiopulmonary insufficiency and high altitudes. The results in substantial increase in amount of oxygen delivered, 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 PO_2 (partial pressure of oxygen) in the lungs remains high enough tha oxygen binding in the lungs is not compromised.

BOHR EFFECT

The increase in acidity of Hb as it binds oxygen is known as Bohr effect. This B.E equivalently is the increase in basicity of Hb as it releases oxygn. Thus, low PH weakens Heamoglobin's oxygen affinity enhancing oxygen delivery.

$$HHb + O_2 = HbO_2 + H^+ \qquad (2.4.1)$$

Thus, increase in proton ion concentration (H^{+}) with favour formation of free oxygen from HbO_2 and conversely that oxygenation of Hb will lower the PH of the solutionl. Carbondioxide is closely tied to Hb and to the problem of maintaining a constant PH in the blood. Carbondioxide CO_2 is present in the blood in 3 major forms: (1) Dissolved carbondioxide (2) As HCO_3 formed by ionisation of H_2CO_3 produced when carbondioxide reacts with water.

$$CO_2 + H_2O = H_2CO_3 \qquad (2.4.2)$$

(3) 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. Carbondioxide after it enters th blood stream for transport, generates hydrozonium ion H_3O^{+} in the blood; through H_2CO_3 formation. CO_2 entering the blood diffuses into the erythrocytes. Within the erythrocytes, most of the CO_2 is acted upon by intracellular enzymes - carbonic anhydrase, which catalyses the reaction

$$CO_2 + H_2O = H_2CO_3 \qquad (2.4.3)$$

Because of the compartmentalisation of carbonic anlydrase, 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 HCO_3 is higher than arterial. The absence of carbonicanhydrase in the plasma causes taking up of CO_2 from the blood. This is done by the reaction of carbondioxide with amino group of proteins with the red cells to form carbamino group. Hemoglobin protein is most important in the reaction. The deoxy-Hb forms carbamino-Hb more readily than Oxy-Hb does, and OxyHb causes the release of CO_2 bound in carbamino-Hb. Thus, deoxy-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 heamoglobin causes the release of CO_2 to the atmoshere.

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 limits the number that potentially cna participate in this reaction. Essentially, only the 4 terminal amino groups of Hb chains can form carbomino product. The N-terminal amino group of B-chain forms part of the binding site of DPG, thus competition arises. Carbondioxide diminishes the effect of DPG and DPG in terms diminishes the ability of Hb to form carbamino -Hb, thus the releaase of carbondioxide. 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 carbondioxide to the atmosphere in the lungs where carbondioxide tension is low. Also in the tissue, with high tension of CO_2 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 binding of oxygen to Hb to form OXY-Hb.

B REGULATION OF BLOOD PH

Heamoglobin also plays the major role in handling the H+3 O ion (acid) produced by in CO2 transport. Heamoglobin has 38 Histidine residues 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 carbondioxide transport. The buffer system (the inorganic phosphate buffer the plasma protein buffer and heamoglobin buffer) minimizes the change in Ph that occurs when acid or base is added to the blood. This control 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 enzymic activity.

PHYSICAL FACTORS THAT AFFECTS HEMOGLOBIN'S OXYGEN BINDING

(1) TEMPERATURE

High temperature weakens Hb's oxygen affinity. Temperature has significant effect on oxygen binding by Hb. At below normal temperature, the binding is tighter resulting in increase percentage Hb saturation, thus low partial pressure PO_2 oxygen (PO_2) needed for saturation. At high temperature the binding is weaker, thus higher PO_2 is needed for saturation. High temperature and high level DPG have enhancing unloading of oxygen. The temperature effect is physiologicallyuseful, 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 PO_2 minimizes compromise of O_2 uptake in the lungs under these conditions. The tighter binding of oxygen which occurs in hypothemic conditions is significant in hypothemia induced for surgical purposes. The de-crease oxygen utilization by the body and increase solubility of oxygen in plasma at low temperatures, with the increase solubility of carbondioxide, which acidifies the blood; compesate for Hb's diminished ability to release oxygen.

(2) **PH**

Low PH weakens Hb's oxygen affinity. Low PH (Acidity) enhance oxygen delivery whereas high PH (basicity) increase binding of oxygen to Hb; at the same PO2. Thus, percentage saturation of Hb with oxygen increases with increase in PH. The influence of Ph upon oxygen binding is physilogically important, since a decrease in PH signifies increase in oxygen demand. An increased metabolic rate results in increased production of carbondioxide and as in muscular exercise, lactic acid. Lactic acid also is produced by hypoxic tissue, these acids 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 releases oxygen.

2.3 HEAMOGLOBINOPATHIES

These are many inherited abnormalities of Hb synthesis in which there is formation of a structurally abnormal heamoglobin. They may involve the substitution of one amino acid in one type of polyeptide chain for some other amino acid. Or they may involve absence of one or more amino acid residues of a polyeptide chain, or abnormal duplication of one amino acid or sequence of amino acids. In some cases, the change is clinically insignificant but in orders it causes serious disease. Substitution, delection and 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 hemoglobi as oxygen carrier.

Some abnormal Hbs have altered affinity for oxygen. If oxygen affinity is increased (P50 decreased), oxygen delivery to the tissues will be diminished, unless some sort of compesation occurs. Example Hb Rainier has P50 of 12.9mm Hg about 14 MmHg lesser than that of normal adult Hemoglobin HbA 27 mmHg. The body of person with Hb Rainier responds by producing more red cells - polycythemia and more Heamoglobin. In Hb chesapeake, there is also increase affinity for oxygen and thus decrease delivery of oxygen and thus decrease delivery of oxygen to tissues at low oxygen tension. polycythemia is the body's response to the defect. Methemoglobinemia in which case abnormal amount of methemoglobin is in the blood. Methemoglobin is a form of heamoglobin that combines lastingly (irreversibly) with oxygen rather that temporarily. In Hb kansas, there is diminished oxygen

binding and in other form of heamoglobinopathy, there is normal heme-oxygen interation. However, with formation of a Hb that precipitates readily in hemolytic anaemia, abnormal structures - Heinz bodies are in the red cells and abnormal pigment in urine.

MOLECULAR PATHOLOGY OF HEAMOGLOBIN

Heamoglobinopathy is a molecular disease cause by familiar formation of abnormal heamoglobin. There are over 250 abnormal heamoglobins, having one form of defect in their structure or the other. These defects are pronouced when the parts of heamoglobin affected are critical to the stability of the corporate molecule or to the physiologic functions of heamoglobin. Apart from the sequence and segment affected, certain amino acid substituent in the normal heamoglobin sequence, have peculair physicochemical properties which are vital to normal functioning of the protein. Any form of alteration of such amino acids would eventually lead to pronouced hindering effect on the heamoglobin in the performance of its physiologic function. There are certain sites which are vitally critical for the heamoglobin molecular stability and its function such as inter chanin interaction site, heme pocket, salt bridges, oxygen binding sites etc. Changes to such sites produce molecular defects in heamoglobin which correspondingly result in clinically significant diseases in individuals with them, examples are:-

(1) CHANGES IN SURFACE RESIDUES

These don't usually have serious effect except when the amino acids involved have nique physicochemical properties critically essential to stability and function of heamoglobin. Example is Glutamic acid (Glu) (Relative hydrophobicity = -9.9) which is replaced by valine Val) Relative hydrophobicity = 1.5) in the B6 - position of heamoglobin molecule. This results in sickle cell disease in homozygous inheritance. In contrast, in HbE Lysine (Lys) (Relative hydrophobicity = 4.2) is substituted for Gluatamic acid in B26 position. This produces no clinical manifestation in both homozygous and heteroxygous states. This can be adduced to the fact that the difference between their relative hydrophobicities is not as high as that between Glu and Val and the preference of both amino acids for water. Moreso, Lysine has prefference for water while valine has preference for non-polar sollvent.

(2) CHANGES IN INTERNALLY LOCATED RESIDUES

These often destabilize the heamoglobin molecule. The internal evnironment of the molecule is hydrophobic, being lined by hydrophobic amino acid residues. Thus, a change of any of these residues

to hydrophillic ones produces instability. This is seen in Hb Harmersmith where phenylalamine (Phe) on position B- 142 is replaced by serine (Ser). In Hb Bristol, Valine in position B67 is replaced by Aspartic acid, (Asp) a polar group in contanct with the heme, which partially occludes the heme pocket. This weakens the binding of the heme to the protein by facilitating access of water to the subunits otherwise hydrophobic interior. Heamoglobin also destabilizes by the distruption of elements of its secondary, tertiary or quarternary structures. The instability of Hb Bibba results from substitution of a helix breaking proline (Pre) for Leucine (Leu) in A 136 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 A1 - B1 contact does not significantly dissociate under physiological conditions, but may do so upon structure alteration. This occurs in Hb Philly in which Tyrosine (Try) postion A35, which participates in the H-bonded network; that helps knit together the A1-B1 interface, is replaced by Phenylalamine (Phe).

(3) **CHANGES AT THE BINDING SITES**

Changes stabilizing metheamoglobin: changes at the oxygen binding site, that stabilize the heme in Fe3+ -oxidation state, eliminate the binding of oxygen to the defective subunitts. Such metheamoglobins are designed as HbM and individuals with them are said to have metheamoglobinemia. They have bluish skin, a condition known as cyanosis; which results from the presence of deoxy-heamoglobin in the arterial blood. All known metheamoglobins arise from substitutions that prevents the Fe atom binding with anionic oxygen atom ligand. In Hb Boston, the substitution of Tyrosine (Try) for the distal Histidine (His) in A58 position results in formation of a 5-coordinate Fe^{3+} -complex; with phenolate ion of the mutant Tyrosine displacing inidazole ring of His 87 as the apical ligand. In Hb Milwankee, the A-Carboxyl group of the Glutamic acid (Glu) that replaces valine (Val) i n position B67 forms an ion pair with a 5-coordinate Fe^{3+} complex. Both pheno late and Glutamate ions in these Metheamoglobins so stabilize Fe^{3+} oxidation state that Metheamoglobin reductase is ineffective in converting them to Fe^{2+} form. In HbM 1 wate, Histidine (His) in position A-87 is replaced by Tyrosine (Tyr). Generally, Metheamoglobins have Hill's constant of approximately 1.2, and low cooperativity.

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

4. CHANGES AT THE A1 - B2 CONTACT OFTEN INTERFERE WITH HEAMOGLOBIN'S QUARTENARY STRUCTURAL CHANGES

Most such changes produce heamoglobins, which have increased oxygen affirnity 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 heamoglobin have ruddy complexion. Some amino acid substitution at the A1 - B2 interface results in an increased oxygen affinity. Individuals with such heamoglobins are cyanotic. Amino acid substitution at A1 B2 contact may change the relative stabilities of Heamoglobin's R and T-forms, thereby altering its oxygen affinity. For example, the replacement of Aspartic acid (ASP) at postition B99 by Histidine (His) in Hb Yakima, eliminates the H-bond at the A1-B2 contact that stabilizes the T-form of heamoglobin.

The interloping imidazole ring also acts as a wedge that pushes the subunits apart and displaces them torwards the R-state. This change shifts T=R equiblibrium almost entirely to the R-state, which results in Hb Yakima having an increased oxygen affinity and a total lack of cooperativity. In contrast, the replacement of asparagine (Asn) in position B 102 by Threomine (Thr) in Hb Kansas eliminates the H-bond in the A1-B2 contact that 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 cooperativity.

2.4 SICKLE CELL ANAEMIA

Sickle cell anaemia is one type of heamoglobinopathies caused by a single point substitution of hydropholic amino acid, Valine for hydrophillic amino acid, Glutamic acid in position 6 of the B-chain. The point mutation in the protein sequence brings about drastic physicochedemical and physiological changes resulting in the defects. A few among the physicochemical changes of sickle cell anaemia are lower solubility, decreased electro phoretic movement, 2 units decrease in anionic charge, altered quarternary structure etc. The mutation causes sickle cell heamoglobin (HbS) to aggregate into filaments of sufficient size and stiffness, which deform erythrocytes to a sickle or creacent shape. This is a remarkable example of the influence of primary structure on quarternary

structure.

In every cycle of their journey through the circulatory system, the red cells must squeeze through flexible capillary blood vessels which are smaller in diameterthat they are. In Sickler, many cells assume crescent shape under conditions of low oxygen tension typical of capillaries. The sickling increase red cell rigidity, which hinders their free passage through the capillaries. The sikcle 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 gives rise to extensive tissue damage and excruciating pain. Also, sicklers suffer fom heamolytic anaemia, condition characterised by red cell destruction. This is because the increased mechanical fragility of red cells, halves the normal 120 days life time of these cells.

In case, the sickle cells plug the capillaries of the vital organs in the body such as the brain, the heart, the kidney, the lungs, the 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 dysfunction, respiratory paralysis and spleen infarctions 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 characterised by severe chronic anaemia. This clinical manifestation is punctuated by painful crises which are due to blockade of capillary beds in various organs with masses of red cells. This gives rise to fever and episodic pains in the chest, abdomen or joints that are dificult to distinguish from the effects of other diseases. The blockade of capillary beds in vital organs such as the Brain, Heart, Liver and Kidney prevents the flow of nutrients and oygen 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 3.5-40. 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 interraction which leds to strinkage of the red cells due to oxidation.

III TRANSMISSION OF SICKLE CELL DISEASE

Sickle Cell Anaemia like other heamoglobinopathies and inborn errors of metabolism, is a molecular disease. As such, it is transmissible to progeny by genetic inheritancefrom both parents, the serious and ultimate fatal disease, sickle cell anaemia, is the consequence. The cells of all higher animals but germ cells have 2 homologous copies of each chromosome (diploid) with the exception of sex chromosome (haploid). An organism may be heterozygous or homozygous for a gene, if its cell respectively bears one or two copies of the gene. The heamoglobin of individuals who are homozygous for sickle cell anaemia is almost entirely sickle cell homoglobin Hos. But heterozygous individuals for sickle cell anaemia have Hb that is 40% Hbs. Such carriers of sickle cell trait lead a normal life even though their erythocytes have a shorter life time than those of normal individuals.

In term of genotype, sickle cell gene manifests 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-linked ie it is not bias in term of sex in its manifestation; this means that it has equal chance of occurence in either sex. The normal adult human hemoglobin gene, gene A1 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 heterozygous individuals, the normal gene, gene A1, prevalils over the sickle cell gene. As such thephysiologic properties of normal adult hemoglobin manifests in the blood of heterozygous individuals, though he is harmed relatively little, 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 mendelian inheritance, though the gene is inherited as a mendelian recessive. The following figures illustrate the inheritance of sickle-cell gene and its manifestation as either in carriers or sicklers in the offsprings.

(1) Inheritance in the offspring of Normal and carrier parents

Fig. 2.4.1 Normal A A A S Carrier (Parent genotypes)

A A A S A A AS (Genotypes of the offsprings)

Phenotypes: 50% Carriers, 50% Normal

(2) Inheritance in the offsprings of Normal and Sickler parents

Fig. 2.4.2 Normal A A S S Sickler (Parent Genotypes)

A S A S A S A S (Genotypes of the Offsprings)

Phenotypes: 100% Carriers

(3) Inheritance in the offsprings of carrier parents

Fig. 2.4.3 A S A S Carrier (Parent Genotypes)

A A A S A S S S (Genotypes of the Offsprings)

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

(4) Inheritance in the offspring of a carrier and a sickler parents.

Fig. 2.4.4 Carrier A S S S Sickler (Parent Genotypes)

A S A S S S S S (Genotypes of the offsprings)

Phenotype 50% Carrier, 50% Sickler

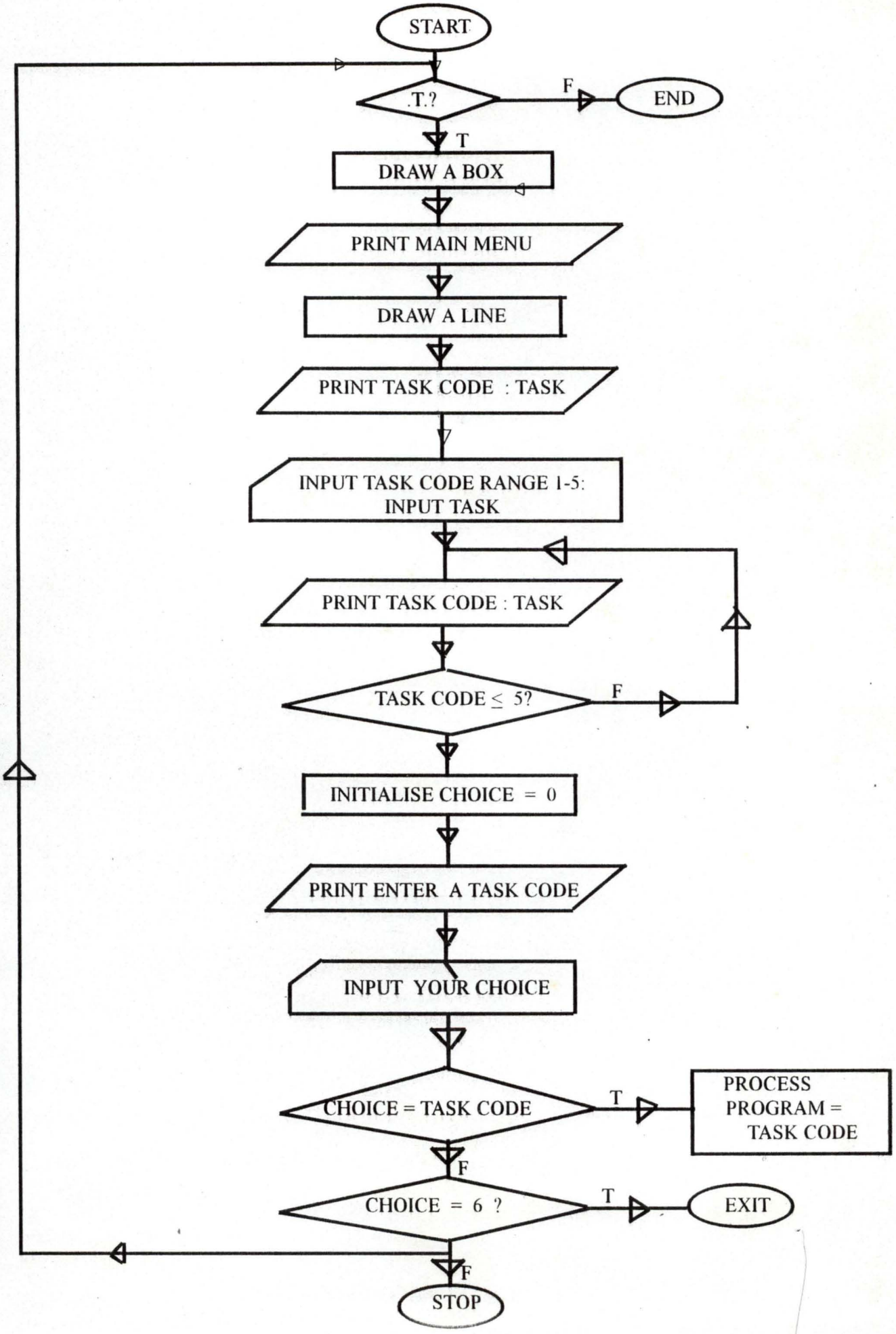
Heterozygous individuals (ie those with AS genotype) or carriers may have upto 50% of their heamoglobins as Hbs while the other 50% is HbA. They exhibit symptoms of sickle-cell anaemia under extreme hypoxia.

IV	HEAMOGLOBIN GENOTYPE IN RELATION TO S.C.A.	-	Heamoglobin genotype occurs in three characrteristics in relation to SCA; Normal heamoglobin genotype AA, carrier heamoglobin genotype AS and sickle-cell genotype SS
V	SEX AND INHERITANCE	-	Genetic inheritance of Sickle Cell trait is not sex linked ie not bias to either sex .This means that both sexes are susceptible. Phenotype manifestation of the sickle cell trait is also not sex - linked .
VI	FEATURES	-	Life span of red cells of sicklers is much shorter , ranges between 17 and 60 days than a average life of normal red cells (120 days).

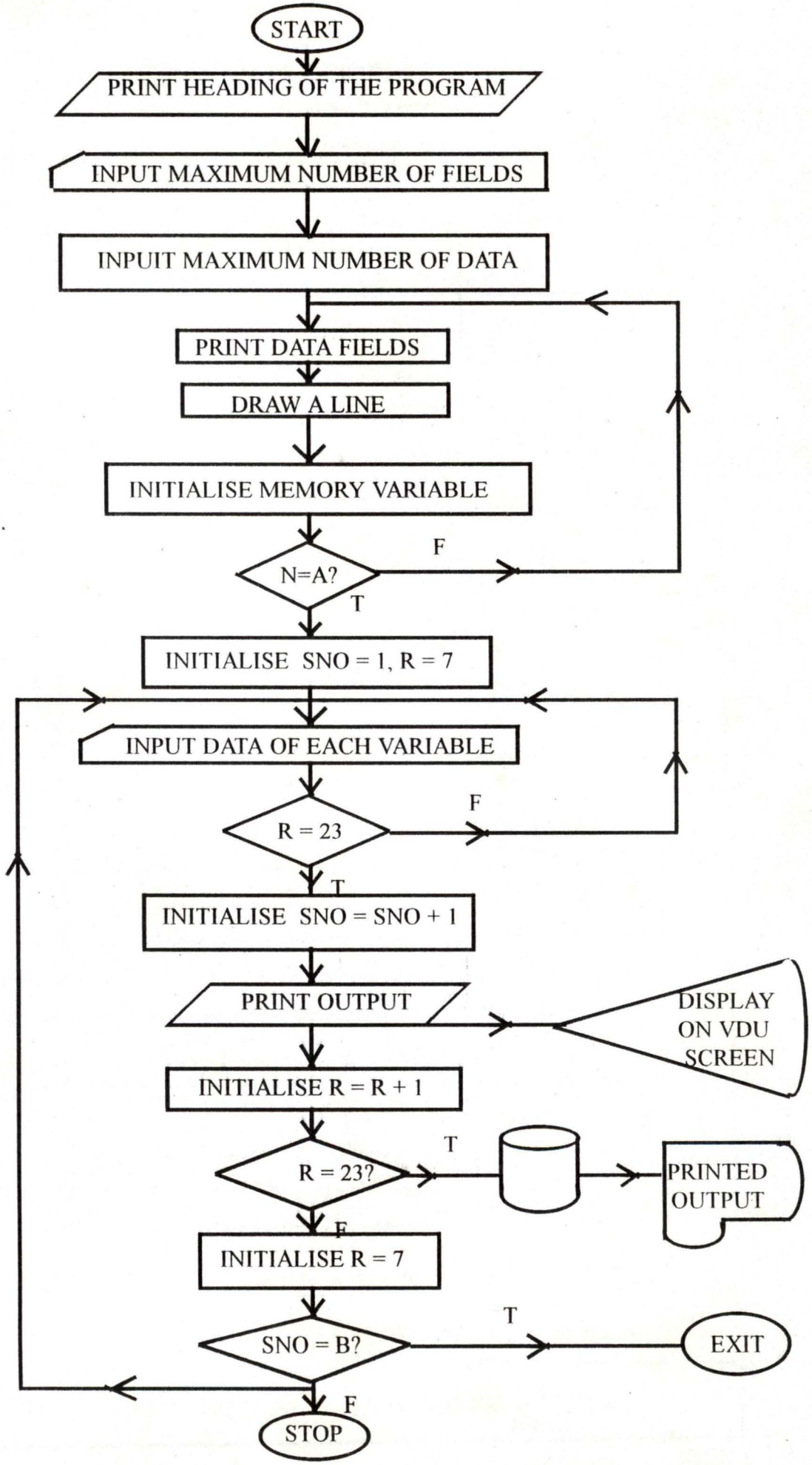
			SCA (HBS) HAS decreased Solubility in the deoxygenated state (Low oxygen ten sion) polymerisation of deoxy -Hb chains to form stiff or rigid filaments which extends throughout the lenght of the cell. (Gelation) . Deformation of red cells crescent or sickle shape. Blockade of capillary beds by masses of deformed red cells . Fragility of red cells and heamolysis. HBS has low oxygen affin ity T state of HBS is stabilized over R state.
(VI)	MANAGEMENT	-	By blood transfusion . U se of erythropoeitic drug -ferrous salt Vitamins,Folic Acid.

CHAPTER THREE

SOFTWARE ALGORITHMS FOR HEAMOGLOBIN PROTEIN ANALYSIS



REPRESENTATIVE FLOWCHART ALGORITHM FOR THE HUMAN HEAMOGLOBIN PROTEIN PROGRAMS



HEAMOGLOBIN PROTEIN SYSTEM EXPERIMENTATION SOME ABNORMAL HUMAN HAEMOGLOBINS RESULTING FROM POINT MUTATIONS IN THE

4.2 GENES FOR A,B,C, OR D CHAINS

S/NO	NAME	RESIDUE	SUBSTITUTION	MAJOR ABNORAML PROPERTY
1.	Hb1	A16	Lys-Glu	None
2.	HbG Honolulu	A30	Glu-Glu	None
3.	Hb Torino	A43	Phe-Val	0_2 affinity ID
4.	Hb Hasharon	A47	Asp-His	Unstable
5.	HbM Boston	A58	His-Thr	0_2 affinity
6.	HbJ Buda	A61	LYs-Asn	0_2 affinity
7.	HbA Pest	A74	Asp-Asn	None
8.	HbM Iwate	A87	His-Thr	Met Hb, 0_2 affinity I
9.	Hb Rampa	A95	Pro-Ser	Dissociation I
10.	Hbj Tangariki	A115	Ala-Asp	None
11.	Hb Bibba	A136	Leu-Pro	Dissociation
12.	Hb Mosaka	A58	His-Tyr	Methaemoglobinemia
13.	Hb Quong Sze	A125	Leu-Pro	
14.	Hb Chesapeake	A92	Arg-Leu	0_2 affinity I
15.	HbC	B6	Glu-Lys	None
16.	HbS	B6	Glu-Val	Sickling, 0_2 affinity I
17.	Hbj Baltimore	B16	Gly-Asp	None
18.	HbE	B26	Glu-Lys	None
19.	Hb Genova	B28	Leu-Pro	0_2 affinity I
20.	Hb Tacoma	B30	Arg-Ser	Bohr effect I
21.	HbM Hammer smith	B42	Phe-Ser	Unstable, 0_2 affinity I
22.	HbM Zuriah	B63	His-T yr.	Unstable, 0_2 affinity D
23.	Hbm Saskatoon	B63	His-Try	Meth
24.	Hbm Hyde park	B92	His-Tyr	Meth b
25.	HbA Kolin	B98	Val-Met	Unstable 0_2 affinity D
26.	HbD Punjab	B121	Glu-Asn	0_2 affinity I
27.	Hb Abruzzo	B146	His-Arg	0_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 DELETED RESIDUES, EXTENDED SEQUENCES OR SEQUENCES RESULTING FROM CHAIN DUPLICATION

TYPE OF MUTATION	NAME	STRUCTURE	FUNCTIONAL ABNORMALITY
DELETION	Hb Leiden	B6 or B7	Unstable
	Hb Tochigi	B56 - B57	0_2 affinity I
		(Gly-Asn-Pre-Lys)-0	Unstable
	Hb Gum Hill	B91 - B95	Unstable
	Hb Conventry	B141 Leu - 0	
		(Leu - His - Cys -Asp -Lys) - 0	0_2 affinity
EXTENDED	Hb Constant Spring	B141 Arg in A - chain is not carboxyl terminal and chain extended for 31 additional residues	
	Hb Tak	B146 is not carboxyl terminal and chain extended for 10 additional residues	02 affinity I
	Hb Koya Dora	A 141 Arg not carboxyl terminal and chain extended for 16-17 additional residues	
FRAME SHIFT	Hb Wayne	A 131-141 Frameshift in codons to give the sequence. Thr-Ser-Asn-Thr-Val-Lys-Leu -Glu-Pro-Arg-CooH at carboxyl terminus.	
INSERTION	Hb Grady	A 118-A119, 3 residues inserted between B 118Thr and B 119 Pro	
FUSION	Hb Leprore	First third of sequence like G-chaine	

4.4 PHYSIOLOGIC ASPECTS OF SOME ABNORMAL HAEMOGLOBINS

HAEMOGLOBIN	RES	SUBS	REGIONS	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-Asn	A1-B2	1	N	1.1	~20	-
4. Hb Rad cliffe	B100	Asp-Ala	A1-B2	12	N	1.1	~20	N
5. Hb Brigham	B100	Pro-Leu	A1-B2	19.6	N	D	16-19	N
6. Hb Denmark Hill	A95	Pre-Leu	A1-B2	1	-	1.8-2.4	~13	-
7. Hb San Kansas	B109	Val-Met	A1-B2	16.4	N	~20	~17	N
8. Hb Kansas	B109	Asn-Thr	A1-B2	~7.0	N	~1	~14	-
9. Hb Rainier	B145	Tyr-Cys	SALT	12.9	N	1.1	16.20	-
10. Hb Andrew M	B144	Lys-Asn	SALT					
11. Hb Syracuse	B143	His-Pro	DPG	11	D	N	~20	N
12. Hb Rahae	B82	Lys-Thr	DPG	18	N	N	~19	D
13. Hb Providence	B82	Lys-Asp Or-Asn	DPG	D	D	2.5-2.7	-	DD
14. Hb Hearthrow	B102	Phe-Leu	HEME	1	N	~1	16-21	-

The P_{50} of 0_2 required for saturation of whole blood containing the abnormal haemoglobin. For normal adult haemoglobin in whole blood it is 27 $\pm$ 2 mmHg at PH 7.4 and physiologic temperature of 37^{0}c. Conc in Hills equation for normal value of normal whole blood is 2.8 $\pm$ 0.2, Conc in Hill equation = Normal adult values are 14 $\pm$ 2 for females and 16 $\pm$ 2 for males.

Res = Residue, Subs = Substitution, Region = Region of molecule Affected, P50 = P50 of Oxygern, BE = Bohr Effect, Hill = n< in Hills Equation, Conc = Concetration in g/100ml, DPG = DPG Interraction, Salt = Salt Bridges, DPG = DPG Site.

CHAPTER FIVE
CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The exact descri ption of a protein structure in term of its primary, scondary, tertiary and quarternary structures, determines the specific chemical and biological properties of the protein in the native state. Similarly, genetic traits are expressed through the synthesis of proteins which play dynamic or structural roles that are 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 proteins 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 categorised into classes: (a) The life-tolerant ones (b) The life compatible (c) The fatal ones and (d) Those that pose constant threats to good health - diseases.

Among the first class are HbD punjab, Hb' Abbruzzo, Hb Bethesida and many others, while HbC, HbI, 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 metheamoglobinemia. Those structural or functional abnormalities resulting in diseases are HbC Harlem, 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 haemoglobinopathies.

For now, genetic diseases defile any curative measure, it is hoped however, that with the advancement of genetic technology, tommorrow may see the technical possibility of "gene therapy" which may serve as panacea to certain human genetic diseases, 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 familial diseases in experimental animals so that they can be studied more carefully. For the genetic therapy to be possible there must be insertion of normal genes into human somatic 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 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 characteristics.

5.2 RECOMMENDATIONS

Disease generally are caused by a change in normal or healthy external and internal environments of an organism. The external environment includes the physical, chmical, 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 ill-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 diseases often do not lend themselves to curative measures, at best they can be medically managed to tolerate life. Some degree of prevention can be achieved via health education and genetic counselling. 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 hererozygous inheritance. The genes coding for the traits are oftern recessive to the normal and dominant genes, these facts seriously reduce the number of potential candidates of such diseases.

In cases where the defective genes are dominant over the normal genes, they are not at all compatible with life, except if such functions performed by the proteins are not essential and critical to life. Some homozygous inheritance may be out-rightly fatal and not at all life-compatible e.g homozygote 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 are not curable and there is virtually nothing 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 counselling. Taking the case of sickle cell anaemia as a classical example, and derived from the genetic prospects of the offsprings, it is not genetically advisable for sickle-cell trait carrier pairs to contract a marital 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 remaining 50% concerns with normal children. Worse still, is the phenotype prospect of the offsprings of a sickler and a carrier parents (50% Siklers).

Also important is the knowledge of the environm,ental conditions that are healthy to patients of genetic diseases, or carriers of such disease traits. For instance, sickler are not compatible with cold weather (Low temperatrure precipitates sickiling). 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 polluted air is detrimental to, or precipitates sickling. There is also the need for regular use of erythropoetic drugs and vitamins to compensate for loss during haemolysis. Stress induces metabolic rate of the body and this leads to decrease in PH (acid-base measurement) i.e. acidity increases. Low ph or high acidity wekens the binding of oxygen with the Oxygen carriers protein - haemoglobin, although it at the same time enhances oxygen with the haemoglobin oxygen complex to the tissues. However, since stress requires increase 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 oow temperature, 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 temperature. Thus, the oxygen-delivery in hypothermic condition does not commensurate to oxygen need of the body. Moreso, there is decrease in metabolic rate of the body and thus corresponding decrease in prodution of carbondioxide and latic acid. Since oxygen delicery increases with increase in acidity, hypothemia does not augur well physiologically, for sicklers. In the contrary, high temperature weakens Hb's oxygen affinity implying decreased saturation of Hb at constant partial pressure of oxygen in the inhaled air. However, high temperature has enhancing unloading of oxygen to the respiring tissues. Consistent with this, is the fact that at high

temperature, there is increased metabolic rate requiring a rise in oxygen delivery from the oxygen-haemoglobin complex, yet the percentage of oxygen delivered coupled with the decreased saturation of sickle haemoglobin with oxygen in pyrexic or malarial 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 linearly proportional to their partial pressure. At high and low altitudes, there are decreased and increased solubilities respectively of these gasses in the inhaled air. Decreased 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 aggrivate the hypoxic of sickling and may be fatal. In low altitudes, there 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 indpendent of external partial pressure of the inhaled gases, but rather depends on intrinsic property of the haemoglobin. thus, sicklers are not at physiologic advantage at low altitude. Rather on returing to the normal atmoshperic pressre, 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 anti malarials and other drugs are not desirable for sicklers, since they produce oxidative haemolysis of red cells resulting in anaemia; in Glucose-6-phosphate dehdrogenase - defficient individuals. Sicklers may not be necessarily deficient in this reductive enzyme (which reduce hydrogen peroxide produced from metabolic reactions, that causes lysis of red cell), but it is safter to avoid this oxidising drugs, which not only precipitates but also aggravates anaemia in sicklers. Sickle cell trait carriers do not experience the phenotypic manifestations of the defective gene, however, under extreme hypodia, the symptoms of sickle cell amaenia may set in. This is due to inherent component of the defective gene in the haemoglobin genotype of carriers.

Finally, there is a need for individuals to know 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

Alleles	Alternative forms of a gene
B.E	Bohr effect, the increase in acidity of haemoglobin as it binds Oxygen
CO2	Carbondioxide gas ; or a molecule of carbondioxide
DPG	Diphosphoglycerate
Diploid	A cell that contains 2 chromosomes 2N of each type
Electrophoresis	is a separation technique for electrically charged substances ,the principle of operation is based on the netchange on the substance
Fe^{2+}	Iron atom in 2- oxidation state
GABA	Gamma amino butytric acid, a chemical neurotransmmitter
G.6.PD	Glucose -6-phosphate dehydrogenase
Genome	The total genetic content of a cell
Genotype	The genetic characteristics of an organism distinguished from its observable characteristics or phenotype
Haploid	A cell containing only one chrosome of each type
H b	Haemoglobin ,respiratory transport protein in the blood
HBO_2	Oxygenated haemoglobin or oxy - haemoglobin or oxy -heme
HHB	Deoxygenated haemoglobin or de oxy - haemoglobin
HBA	Human adult haemoglobin
HBS	Sickle cell haemoglobin
Isoelectric point	The PH at which a protein has no net charge
Keq	Equilibrium constant for a reversible reaction
Mb	Myoglobin , respiraory transport protein in the muscle
NMR	Nuclear magnetic resonance
O_2	Oxygen molecule
P H	Acid - base measurement
Phenotype	The observable trait (s) that result from the genotype in cooperation with the environment
PK_A	Negative logarithm of the ionisation constant of a acid
PO_2	Patial pressure of oxygen
P_{50}	Partial pressure corresponding to 50% saturation
RBC	Red blood cell (red cell) or erythrocyte
SDS	Sodium dodecyl sulphate (Detergent)
TLC	Thin layer chromatography
WBC	White blood cell or leucocytes

APPENDIX II

HUMAN HAEMOGLOBIN PROTEIN SOFTWARE PROGRAMS

```
SET TALK OFF
SET STATUS OFF
SET SCOREBOARD OFF
CLEAR
@1, 31 TO 3, 44
SET COLO TO W + /B
@2, 32 SAY "ITRODUCTION'
SET COLO TO  W+
@6, 13 SAY 'THESE PROGRAMS ARE DESIGNED AND WRITTEN AS PART OF'
@7, 13 SAY "PROJECT SUBMITTED TO THE DEPARTMENT  OF MATHEMATICS"
SET COLO TO B+
@10, 39 SAY "BY"
ST COLO TO W+/R
@12, 29 SAY "OYEKALE OLADOKE ISSAC"
SET COLO TO W
 @13, 33 SAY "(B.Sc BIOCHEM)
@14, 33 SAY "PGD/MCS/084/96"
SET COLO TO W+
@16, 13 "IN PARTIAL FULFILMENT OF THE  REQUIREMENT FOR THE"
@17, SAY "AWARD OF POST GRADUATE DIPLOMA IN COMPUTER SCIENCE"
@ 18, 13 SAY "OF THE FEDERAL UNIVERSITY OF TECHNOLOGY , MINNA"
@20, 33 SAY "MARCH, 1997."
SET  COLO TO
@21, 1 SAY" "
@22, 1 SAY " "
CLEAR
@1, 29 TO 3, 50
SET COLO TO W+ /B
@2, 30 SAY "TOPIC OF THE PROJECT"
SET TO COLO TO G+
@5, 5 SAY "APPLICATION OF COMPUTER IN THE DETERMINATION OF PRIMARY
                STRUCTURE OF
@6, 5 SAY "HUMAN RED CELL PROTEIN - HAEMOGBLOBIN."
SET COLO TO W+
@8, 5 SAY "THE STUDY CONCERNS WITH STRUCTURE-FUNTION RELATIONSPHIP
IN THE HAEMO
@9, 5 SAY "GLOBIN MOLECULE AND POINTS OUT VARIOUS ABONORMALITIES AND
```

```
@10, 5THEIR CONSEQUENCES ARISING FROM CHANGES IN PRIMARY STRUCTURE
@11, 5(SEQUENCE OF AMIN O ACIDS) OF THE TRANSPORT PROTEIN."
SET COLO TO N
@12, 1 TO 22, 1
SET COLO TO
WAIT
OPTION = 0
DO WHILE .T.
CLEAR
MAIN MENU
SET COLO TO G+
@5, 10 TO 19, 70 DOUBLE
SET TO W+/R
@4, 35 SAY "MAIN MENU"
SET COLO TO W+
@6, 12 SAY "1 Amino Acid Sequences of Adult Human Haeglobin. "
@8, 12 SAY " 2. Some Abnormal Human Haemoglobin Resulting From Point"
@9, 12 SAY " Mutations in The Genes For alpha, Beta, Gamma Or"
@10, 12 SAY " Delta - Chains."
@12, 12 SAY "3. Some Abnormal Haemoglobins With Delected Residues,"
@13, 12 SAY " Extended Sequences Or Sequences Resulting From"
@14, 12 SAY " Chain Duplication."
@16, 12 Say "4. Physiologic Aspects of some Abnormal Haeglobins. "
@18, 12 SAY " 0 OUIT,"
@20, 23 say " ENTER OPTION [1 - 4] OR 0 TO QUIT "GET OPTION PICT "9" RANGE READ
DO CASE
CASE OPTION = 1DO AMONI
CASE OPTION = 2
 DO MUTATION
CASE OPTION = 3
DO ABHB
CASE OPTION = 4
 DO PHYSIO
CASE OPTION = 0
CLEAR
EXIT
ENDCASE
ENDDO
RETURN
```

```
@       1,15 say "SOME ABNORMAL HUMAN HAEMOGLOBIN RESULTING FROM PINT"
@       2,15 say MUTATIONS IN THE GENES FOR AIBID OR G-CHAINS"
@       3,15 to 3,50 Double
@       5,1 say "S/NO"
@       5,5  say "NAME OF HAEMOGLOBIN"
@       5,87 say "RESIDUE CHAIN"
@       5,44 say "SUBSTITUTION"
@       5,60 say "MAJOR ABNORMAL"
@       6,65 say "PROPERTY"
@       7,1 to 7,74
@       3,5 to 3,70 double
@       4,1 say "S/NO
@       4,5 say "TYPE OF"
@       4,16 say 2 SUIB"
@       4,20 say "NAME"
@       4,41 say "STRUCTURE"
@       4,66 say FUNCTIONAL
@       5,5 say MUTATION"
@       5,16 say "CLASS"
@       5,66 say "ABNORMALITTY"
@       6,1 to 6.76
R       =       7
DO WHILE NOT EOF ( )
@       R 3,2 say SNO
R       5 say MUTATION
R       17 say SUBCLASS
R       20 say NAME
@       R.,41 say STRUCTURE
@       R.,66 say ABFUN
R       = R+1
SKIP
IF R =  24
WAIT
@       7,0 clear 24,79
R  =  7
ENDIF
ENDDO
RETURN
R  =  8
```

```
DO WHILE, NO EOF ( )
@       R, 2 say SN
@       R, 5 say NAME
@       R, 27 say RESIDUE
@       R, 44 say SUBS
@       R, 58 say MAJOR
R   = R  +   1
SKIP
IF R  =  24
WAIT
@       7,0 clear 24,79
R   =  7
ENDIF
ENDDO
RETURN
R  =  8
DO WHILE , NOT. EOF ( )
@       R, 2 say SN
@       R, 5 say NAME
@       R, 27 say RESIDUE
@       R, 44 say SUBS
@       R, 58 say MAJOR
R   = R  +   1
SKIP
IF R = 24
WAIT
@       7,0 clear 24,79
R   =  7
ENDIF
ENDDO
RETURN
R  =   8
DO WHILE.  NOT.  EOF ( )
@       R,2 say SN
@       R, 5 say NAME
@       R,27 say RESIDUE
@       R,44  say SUBS
@       R,58 say MAJOR
```

```
R = R + 1
SKIP
IF R = 23
WAIT
@ 8.0 clear to 24,79
R = 8
ENDIF
ENDDO
RETURN
SNO = 1 AHb. PRG
USE AHb
DO WHILE SNO = 5
Append blank
@ 1, 5 say S/NO" get SNO
@ 2, 5 say "Type of mutation" get MUTATION
@ 3, 6 say "SUBCLASS" get SUBCLASS
@ 4, 5 say "NAME" get NAME
@ 6, 5 SAY "STRUCTURE" get STRUCTURE
@ 7, 5 SAY "FUNCTIONAL ABNORMALITY" get ABFUN
READ
SNO = 1
ENDDO
CLEAR
@ 1,5 say "SOME ABNORMAL HAEMOGLOBIN WIHT DELECTED RESIDUES OR
EXTENDED RESIDUES"
@ 2,5 say "OR RESIDUES RESULTING FROM CHAIN DUPLICATION"
PHYPRO
S = 1
USE PHY
DO WHILE S = 14
Append blank
@ 3,10 say "S/NO" get S
@ 3,10 say "HAEMOGLOBIN" get Hb
@ 4,10 say "REDIDUE" get RESIDUE
@ 5,10 say "SUBSTITUTION" get SUBS
@ 6,10 say "REGION IN MOLECULE AFFECTED" get REGION
@ 8,10 say "BOHR EFFECT" say BOHR
@ 9,10 say "N = IN HILL'S EQUATION" get HILL
@ 10,10 say "CONC (E) g/100 ml" get CONC
```

```
@       11,10 say "DPG INTERACTION" get DPG
@       13,15 say "COMMENT" get COMMENT
READ
S =   S +  1
ENDDO
CLEAR
@       1,15 say "PHYSIOLOGIC ASPECTS OF SOME ABNORAML HAEMOGLOBIN"
@       2,15 to 2, 65 double
@       4, 0 say "S/NO
@       4,4 say "NAME OF"
@       4,25 say "REST"
@       3,28 say "SUBSTITUTE"
@       4,39 say "REGION IN"
@       4,47 say "P50 OF"
@       4,53 say "BOHR"
@       4,49 say "N =  IN
@       4,64 say "CONC (E)
@       4,72 say "DPG INT"
@       5,4 say "HAEMOGLOBIN"
@       5,25 say "-DUE"
@       5,29 say "-TION"
@       5,38 say "MOLECULE"
@       5,47 say "OXYGEN"
@       5,53 say "EFFECT"
@       5,29 say "HILL'S"
@       5,64 say "9/100"
@       5,72 say "REACTION"
@       6,38 SAY "AFFECTED"
@       6,59 say "EQUATION"
@       6,65  say "ML"
@       7,0 TO 7, 79
R =   8
DO WHILE. NOT. EOF ( )
@       R, 1 say S
@       R,4  say Hb
@       R, 25 say RESIDUE
@       R, 29 say SUBS
@       R, 38 say REGION
```

```
@       R, 47 say OXYGEN
@       R, 53 say BOHR
@       R, 59 say HILL
@       R, 64 say CONC
@       R, 72 say DPG
R  =  R + 1
SKIP
IF R = 18 OR R = 16
@       19,1 say COMMENT
ENDIF
IF R = 24
WAIT
@       8,0 clear to 24,79
R  =  8
ENDIF
ENDDO
RETURN
```

```
cls
for x=1 to 25:color 1,0:locate x,1:?string$(80,219);:next
for x=3 to 22:color 4,1:locate x,4:?string$(74,219);:next
locate 8,6:color 15,4:?"THIS PROGRAM IS DESIGNED AND WRITTEN
AS PART OF THE PROJECT SUBMITTED"
locate 10,6:?"TO THE DEPARTMENT OF MATHEMATICS/COMPUTER
SCIENCE BY OYEKALE OLADOKE"
locate 12,6:?"JSAAC (B.SC BIOCHEM) PGD/MCS/084/96 IN PARTICAL
FULFILMENT OF THE "
locate 14,6:?"REQUIREMENT FOR THE AWARD OF POST-GRADUATE
DIPLOMA IN COMPUTER"
locate 16,6:?"SCIENCE OF THE UNIVERSITY OF TECHNOLOGY, MINNA.
MARCH 1997."
'FOR X= 19 TO 21:COLOR 1,4:LOCATE X,10:?STRING$(30,219);:NEXT
locate 20,26:color 15,1:?"PRESS ANY KEY TO CONTINUE"
a$=input$(1)
screen 12,0,0
gosub fchem:
locate 10,2:?"1":locate 10,5:?"GLYCINE":locate
10,16:?"Gly":locate 10,23:?"2.3":locate 10,29:?"9.8":locate
10,37:?"--":locate 10,44:?"5.97"
locate 10,53:?"0":locate 12,63:?"H—C—C--OH":locate
11,66:?"|":locate 10,66:?"H"
locate 13,66:?"|":locate 13,69:?"‖":locate 14,66:?"NH":locate
14,69:?"O":'locate 15,68:?"2"
locate 16,5:?string$(75,196)
draw "bm536,224 c15,e2,r2,f2,g4,r4
locate 18,2:?"2":locate 18,5:?"ALANINE":locate
18,16:?"Ala":locate 18,23:?"2.3":locate 18,29:?"9.9":locate
18,37:?"--":locate 18,44:?"6.00"
locate 18,53:?"0.5":locate 20,63:?"H C—C——C—OH":locate
19,68:?"|":locate 18,68:?"H"
locate 21,68:?"|":locate 22,68:?"NH":locate 21,72:?"‖":locate
22,72:?"O"
'2
draw "bm555,350 c15,e2,r2,f2,g4,r4
'3
draw "bm503,314 c15,e2,r2,f2,g4,r4,d3,g2,l3
a$=input$(1)

gosub fchem:

locate 10,2:?"3":locate 10,5:?"VALINE":locate
10,16:?"Val":locate 10,23:?"2.3":locate 10,29:?"9.7":locate
10,37:?"--":locate 10,44:?"5.96"
locate 10,53:?"1.5":locate 12,61:?"H C—C—C—C--OH":locate
11,66:?"|":locate 10,66:?"H"
locate 13,66:?"|":locate 13,70:?"|":locate 14,65:?"CH":locat
14,69:?"NH":locate 13,73:?"‖":locate 14,73:?"O"
locate 16,5:?string$(75,196)
draw "bm488,185 c15,e2,r2,f2,g4,r4,d3,g2,l3
draw "bm530,218 c15,e2,r2,f2,g4,r4,d3,g2,l3
draw "bm560,218 c15,e2,r2,f2,g4,r4
locate 18,2:?"4":locate 18,5:?"LEUCINE":locate
18,16:?"Leu":locate 18,23:?"2.3":locate 18,29:?"9.7":locate
18,37:?"--":locate 18,44:?"5.98
locate 18,53:?"1.8":locate 20,6?"H C-C-CH -C—C-OH":locat
19,66:?"|":locate 18,66:?"H"
```