maximal stimulation was caused in general by uridylic and cytidylic acid–containing copolymers. Stimulation by other polymers may be due to contamination with several amino acids of the C^{14}-leucine used and is assumed not to be meaningful.

From the observed incorporation of an amino acid relative to that of phenylalanine with a given polymer and the calculated frequency ratio of UUU triplets to other triplets in this polymer, triplet code letters (of as yet unknown nucleotide sequence except for UUU in the case of phenylalanine) have been assigned to eleven amino acids. The proposed code letters are in excellent agreement with amino acid replacement data in nitrous acid mutants of tobacco mosaic virus.

Note added in proof: The assignment of the following additional code letters will be shown in a subsequent article in these PROCEEDINGS: arginine 1U 1C 1G, glycine 1U 2G, and tryptophan 1U 2G. These were obtained by increasing the sensitivity of the testing method.

Our thanks are due to R. C. Warner and Jo-Yun Chen for the sedimentation cofficients of the synthetic polynucleotides, to Yoshito Kaziro for calling our attention to the review by Tsugita[6] and for the help with the Japanese text, and to Albert Lenny for skillful technical assistance. We are much indebted to W. H. Stein, The Rockefeller Institute, New York, for highly purified samples of L-isoleucine and L-phenylalanine.

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[2] Abbreviations: The capital letters A, U, C, and G are used for the nucleotides adenylic, uridylic, cytidylic, and guanylic acid, respectively, or their corresponding residues in polynucleotide chains: ADP, UDP, CDP, and GDP, the 5′-diphosphates of adenosine, uridine, cytidine, and guanosine.

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INDUCTION OF ALTERED GLOBIN SYNTHESIS IN HUMAN IMMATURE ERYTHROCYTES INCUBATED WITH RIBONUCLEOPROTEIN, †*

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It is now generally assumed that information governing protein synthesis is present in deoxyribonucleic acid (DNA) and that this information is mediated by ribonucleic acid (RNA). It is currently postulated that information is recorded in the specific sequence of bases in DNA and RNA and that this arrangement of bases controls the sequential arrangement of amino acids during protein synthesis, thereby conferring specificity.[1, 2] Studies in the field of microbial genetics have demon-

strated that genetic changes or altered information transfer within bacteria may be induced by such phenomena as transformation, transduction, or by viral infection.[3-8] Infectious DNA and RNA, capable of directing viral information, can be isolated from plant and mammalian viruses and have been the subject of recent investigations.[9] The transfer of information for the synthesis of a specific protein has not as yet, however, been clearly demonstrated in mammalian systems.

The abnormal hemoglobins provide an approach to such studies in human cells. They are at present the only well-studied system in human biochemical genetics where it can be shown that mutations produce chemical alteration in a protein molecule. Ingram has shown that sickle cell hemoglobin differs from normal hemoglobin by the substitution of one amino acid (valine for glutamic acid) in a specific peptide of the β polypeptide chain.[10-12] Abnormal amino acid sequences have now been shown for a number of hemoglobins[13-16] and might, therefore, serve to indicate that specific genotypic alteration has occurred.

This study was undertaken to investigate the possibility that altered protein synthesis might be produced in human immature erythrocytes by exposing them to nucleoprotein containing different genetic information. Immature erythrocytes, capable of synthesizing protein, were incubated *in vitro* with nucleoprotein isolated from the marrow of individuals with genetically different hemoglobin. Altered globin formation was demonstrable in hemoglobin solutions prepared from these cells. This altered globin production was found to depend upon the presence of ribonucleoprotein closely associated with DNA during isolation.

Materials and Methods.—Isolation of nucleoprotein: Nucleoprotein preparations were obtained from marrow of patients with sickle cell anemia, from normal marrow, and from normal spleen. Procedures were designed initially to isolate DNA under mild conditions so as to retain the material in its undenatured state. Marrow was obtained in most instances by aspiration and the plasma removed by centrifugation at 30,000 *g* for 20 min at 0°C. Occasionally marrow was obtained from a rib removed during surgery. Marrow obtained from ribs, sternum, and vertebrae within 2 hr post mortem was also found to be suitable material. The cells or marrow particles were suspended in 100 ml of cold 0.05 *M* sodium citrate and homogenized with a Ten Broeck glass homogenizer. The suspension was agitated with a magnetic stirrer for 60 min at 4°C to permit further lysis of cells. 11.6 gm of NaCl was then added, the material stirred at 4°C for an additional 60 min and the resulting viscous material centrifuged at 30,000 *g* for 30 min at 0°C. The supernatant was dialyzed against 4 liters of 0.1 *M* NaCl and 0.05 *M* sodium citrate for 12 hr at 4°C. The sediment from the first marrow extraction was resuspended with 50 ml of cold 2 *M* NaCl, agitated with a magnetic stirrer for 60 min at 4°C, and centrifuged at 30,000 *g* for 30 min at 0°C. The supernatant from this second extraction was also dialyzed against 0.1 *M* NaCl and 0.05 *M* sodium citrate for 12 hr at 4°C.

After dialysis a clot or gel, containing the majority of the DNA, formed within the dialysis bag if the DNA concentration was sufficiently high. This material was sedimented by centrifugation at 30,000 *g* for 20 min at 0°C, suspended in cold 2 *M* NaCl with the aid of a glass homogenizer, and allowed to stand at 0°C for 18 hr, prior to deproteinization procedures. If no gel formed in the dialysis bag or if the remaining material was still viscous, the nucleoprotein was isolated by slowly adding the liquid to 3 volumes of cold 95 per cent ethanol and removing the fibrous precipitate by stirring rapidly with hooked glass rods. The fibers were dried by suction on a filter pad using a Buchner funnel and then dissolved in cold 2 *M* NaCl with the aid of a glass homogenizer.

After 18 hr, sodium desoxycholate to a final concentration of 0.5 per cent was added; the material incubated at 37°C for 1 hr, and then refrigerated at 0°C for 18 hr. A heavy gelatinous precipitate formed which was removed by centrifugation at 30,000 *g* for 30 min at 0°C. The nucleoprotein was isolated from the supernatant by precipitation in 3 volumes of cold 95 per cent ethanol, dried by suction on filter paper, and dissolved in 2 *M* NaCl with the aid of a glass homogenizer. Occasionally deproteinization with sodium desoxycholate and reprecipitation with 95 per cent ethanol was repeated.

The final product was a slightly opalescent, highly viscous material which was stored indefinitely in 2 *M* NaCl at 0°C without loss of viscosity. Prior to use in an incubation mixture it was dialyzed for 12 hr against 0.15 *M* NaCl at 0°C.

The final product was characterized as follows: DNA content was estimated by the diphenylamine reaction as modified by Burton[17] using thymus DNA preparations as a standard. RNA content was estimated by the orcinol method[18] using d-ribose as a standard. Protein determinations were made by the Folin-phenol method of Lowry[19] using crystalline bovine albumin as a standard. The nucleoprotein preparations prepared in this manner usually contained approximately 80 per cent DNA, 10 per cent RNA, and 10 per cent protein.

Enzymatic degradation of nucleoprotein preparations: DNA degradation was performed by incubating the material with deoxyribonuclease (DNA-ase). 5 μg of 2× crystallized DNA-ase (Worthington Biochemical Corp., Freehold, N. J.) was added per ml of the nucleoprotein preparation in the presence of 0.005 *M* $MgSO_4$ and incubated at 37°C for 30 min. RNA was degraded by ribonuclease (RNA-ase). 5 μg of crystalline RNA-ase (Worthington) was added per ml and incubated at 37°C for 20 min. Protein degradation was achieved by incubating the material with salt-free crystalline trypsin (Worthington), 5 μg per ml, at 37°C for 30 min.

Incubation mixtures: Initially, effects of this nucleoprotein on globin synthesis by immature erythrocytes was determined by incubating it with marrow obtained from patients with proved megaloblastic anemia. The hemoglobin formation of megaloblasts incubated with narrow nucleoprotein (experimental) was compared with that obtained when megaloblasts were incubated without the nucleoprotein (control). Each incubation mixture contained 10 ml of aspirated marrow, 5 ml of tissue culture fluid,[20] 0.1 ml heparin (100 USP units), 0.5 mg folinic acid (0.5 ml), 0.1 mg crystalline B_{12} (0.1 ml), and 10,000 units of crystalline penicillin (0.1 ml). A nucleoprotein preparation obtained from the marrow of a patient with sickle cell anemia was dialyzed against 0.15 *M* NaCl and added to the experimental flask. An equivalent volume of 0.15 *M* NaCl was added to the control. In a typical experiment, the final concentrations in the experimental flask were approximately 100 μg per ml of DNA, 10 μg per ml of RNA, and 10 μg per ml of protein. Incubation was carried out under sterile conditions for 24 hr at 37°C with gentle agitation using flasks loosely stoppered with sterile gauze permitting diffusion of oxygen from room air. Following incubation, cells were sedimented by centrifugation at 2500 rpm and washed 4 times with approximately 10 volumes of 0.9 per cent saline. The cells were then lysed by freezing and thawing 3 times and the stroma removed by centrifugation at 30,000 *g* for 30 min. The resultant hemoglobin solutions were then studied by a variety of procedures for evidence of altered hemoglobin formation.

Similar incubation mixtures utilizing immature erythrocytes from peripheral blood were also tested. Peripheral blood containing 20 to 40 per cent reticulocytes was found to be suitable for study. The cells were incubated with nucleoprotein derived from an appropriate marrow (e.g., obtained from a source with genetically different hemoglobin) and the hemoglobin studied for evidence of altered globin production. No B_{12} or folinic acid was added to these systems. Such incubation mixtures included sickle cell peripheral blood incubated with normal marrow nucleoprotein preparations. Also tested was peripheral blood from patients recovering from blood loss, incubated with nucleoproteins obtained from sickle marrow. The different systems employed are indicated in Table 1.

Isotopic studies: Incorporation of radioactive iron (Fe^{59}) as an index of hemoglobin formation was determined by adding 0.12 μc (80 μg) of ferrous sulfate (Abbott Radiopharmaceuticals, North Chicago, Illinois) to incubation mixtures. The incorporation of amino acids was determined in other incubation mixtures by adding 1.0 μc (1 mg) C^{14}-labeled amino acid to each flask. The amino acids studied (Schwartz Laboratories, Mount Vernon, New York and California Corporation of Biochemical Research, Los Angeles, California) included 1-C^{14}-L-leucine, 1-C^{14}-isoleucine, 1-C^{14}-L-valine, 1-C^{14}-tyrosine, 1-C^{14}-DL glutamic acid, and a C^{14} uniformly labeled amino acid mixture.

Incorporation of Fe^{59} was determined by counting an aliquot of dialyzed hemoglobin isolated from washed erythrocytes in a well-type scintillation counter (Nuclear, Chicago), the counts per minute (cpm) being related to the hemoglobin concentration. Incorporation of the C^{14} amino acids was determined by counting aliquots of hemoglobin eluted from carboxymethylcellulose columns in a gas-flow windowless counter (Nuclear, Chicago). The radioactivity present in these

fractions was expressed as a ratio of the cpm to the optical density (cpm/O.D.) of the hemoglobin at 580 mμ.

Electrophoretic analysis of hemoglobin: The hemoglobins were compared by agar gel, starch gel, and Tiselius electrophoresis. Agar gel electrophoresis was performed by a modification of the technique described by Robinson *et al.*[21] using 1 per cent Ionager No. 2 (Consolidated Laboratories) suspended in 0.05 *M* citric acid–sodium citrate buffer, pH 6.2. After electrophoresis, the gels were stained with benzidine. Starch gel electrophoresis was performed on hydrolyzed starch (Connaught) using a boric acid buffer system, pH 8.6[22] and staining with amido-Schwarz black. Tiselius electrophoresis (Spinco) was performed with samples of hemoglobin dialyzed for 3 days with 3 changes of buffer against sodium phosphate buffer, pH 6.9, ionic strength 0.10.

Carboxycellulose column chromatography: The hemoglobin components derived from the incubation mixture were separated by carboxymethylcellulose (CMC) (Selectacel, standard grade, Schleicher and Schell Co., Keene, New Hampshire) chromatography using columns 13 mm × 60 cm.[23] CMC columns were prepared after placing the CMC in the Na^+ cycle with an equal volume of 1 *M* NaOH and 1 *M* NaCl, adjusting the pH to 6.8, and washing with 500 to 1,000 ml of 0.01 *M* phosphate buffer, pH 6.8. Fifty to 60 mg of the hemoglobin to be analyzed were placed on a column. Elution was carried out at 20°C and was started with 0.01 *M* phosphate buffer, pH 6.8, at a flow rate of 5 to 6 drops per minute, collecting 4-ml fractions in a fraction collector. After the initial components were eluted from the column with the pH 6.8 buffer, a gradient was established with 0.01 *M* phosphate buffer, pH 8.0, using a mixing chamber which contained 50 ml of 0.01 *M* phosphate buffer, pH 6.8. The optical densities (O.D.) of the serial eluate fractions were determined at 580 mμ in a Beckman DU spectrophotometer. Mixtures of sickle hemoglobin and normal hemoglobin can be separated by this technique, the normal hemoglobin being eluted at a lower pH than sickle hemoglobin.

Peptide fingerprint analysis: Hemoglobin fractions eluted from the carboxymethylcellulose columns were concentrated in dialysis bags by contact with Carbowax 20M (Union Carbide Co.) and dialyzed for 72 hr against 0.001 *M* phosphate buffer, pH 7.2, with 3 changes of buffer. Peptide analysis of these hemoglobin fractions was performed by a modification of the methods of Ingram.[12] The hemoglobins were denatured by heating at 90°C for 4 min and an aliquot digested for 2 hr at 37°C with salt-free crystalline trypsin under nitrogen, with constant stirring. The digestion mixtures were immediately lyophilized and then placed in a vacuum desiccator containing KOH and P_2O_5 for 3 days, after which they were reconstituted with 0.5 ml distilled water. Separation of the peptides in two dimensions was then achieved by electrophoresis and ascending chromatography. Electrophoresis was carried out at 650 volts and 20 milliamperes for $3^1/_2$ hr using a pyridine:glacial acetic acid:H_2O buffer (10:0.4:90). Ascending chromatography was carried out for 12 hr using a butanol:glacial acetic acid:H_2O solvent (3:1:1). All samples to be compared were run in pairs under identical conditions.

Results.—Incubation of megaloblasts with nucleoprotein derived from sickle marrow: Heme synthesis in the incubation mixtures was demonstrated by the incorporation of Fe^{59} and protein synthesis by the incorporation of C^{14} amino acids into erythrocytes. The amount of Fe^{59} incorporated ranged from 0.8 to 3.5 per cent of the Fe^{59} added. Since a large excess of C^{14} amino acids was added, no quantitation of incorporation was attempted. However, of the amino acids studied, only 1-C^{14}-L-leucine incorporation was sufficiently great to be useful in assaying distribution of radioactivity among various hemoglobin components.

Hemoglobin solutions derived from incubation mixtures containing sickle marrow nucleoprotein and megaloblasts exhibited abnormal components electrophoretically when compared to control samples. A slow-moving component with a mobility comparable to that of sickle cell hemoglobin was demonstrable on both agar gel and starch gel electrophoresis. This was correlated with an abnormal component present in the experimental but not in the control hemoglobin solutions when compared by Tiselius electrophoresis (Fig. 1). No abnormal electrophoretic components were present in hemoglobin solutions isolated from the incubation mixtures

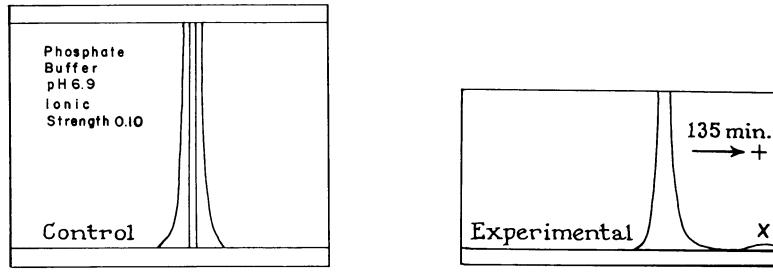

FIG. 1.—Tiselius electrophoretic pattern (Spinco) of hemoglobins derived from megaloblastic marrow incubated with and without nucleoprotein isolated from sickle marrow. A single peak was observed in the control whereas an additional component (x) appeared in the experimental hemoglobin. Two slow-moving components were demonstrable in the experimental hemoglobin but not in the control when samples of these same hemoglobin solutions were compared by starch gel electrophoresis.

at 0, 2, 8, or 12 hr of incubation. Beginning electrophoretic abnormalities were detectable at 18 hr and were readily detectable at 24 hr. Megaloblasts were still in evidence after 18 hr but not after 24 hr.

Separation of an abnormal component from the experimental hemoglobin solutions was achieved by elution from CMC columns (x, Fig. 2). The abnormal component was eluted from the column at a higher pH than the major normal component, resembling sickle hemoglobin in this respect.[23] No such component was present in hemoglobin solutions derived from the control. The elution pattern of hemoglobin

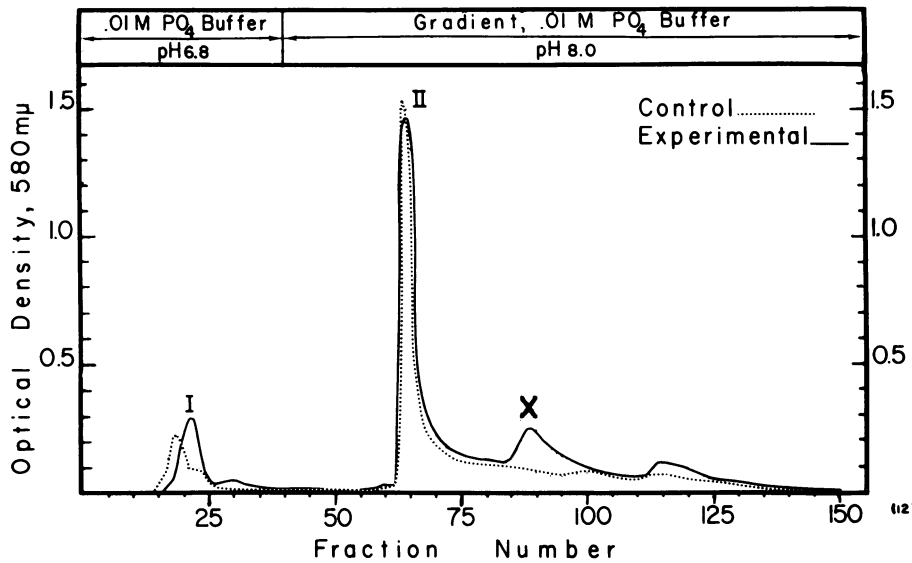

FIG. 2.—Separation of hemoglobin components by carboxymethylcellulose column chromatography. The elution pattern of hemoglobin derived from megaloblastic marrow incubated with sickle nucleoprotein (experimental) is almost identical with that derived from the same marrow incubated without sickle nucleoprotein (control). They differ in that an abnormal component (x) is eluted from the experimental hemoglobin solution without a corresponding peak being eluted from the control. Abnormal components were also demonstrable in the same experimental hemoglobin sample by Tiselius electrophoresis (Fig. 1) as well as by agar and starch gel electrophoresis. The electrophoretic mobility of this abnormal component resembles sickle hemoglobin in its elution from CMC columns at a higher pH than the normal components.

derived from the control and experimental incubation mixtures was identical in all other respects.

Following the addition of 1-C^{14}-L-leucine to the incubation mixtures, the abnormal hemoglobin component was eluted from CMC columns and the amount of radioactivity present in these eluate fractions was compared to that present in other fractions. An increased amount of radioactivity (cpm/O.D.) was present in the abnormal hemoglobin component as compared to other fractions (Fig. 3), indicating an increased net synthesis in this component.

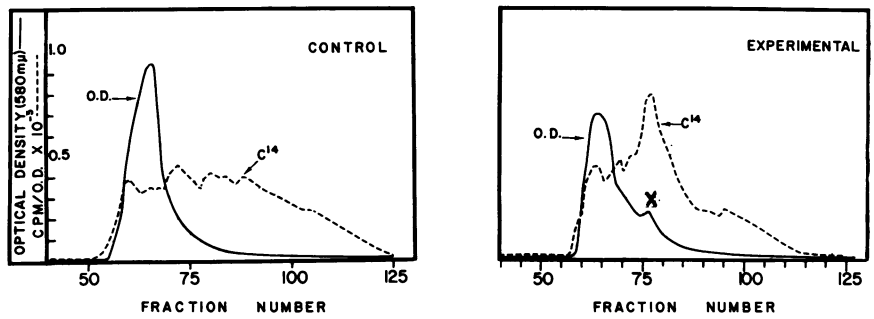

FIG. 3.—Incorporation of 1-C^{14}-L-leucine into various components of hemoglobin separated by carboxymethylcellulose column chromatography. The hemoglobins were eluted by establishing a pH gradient with 0.01 *M* phosphate buffer (pH 6.8 to 8.0). The hemoglobin components separated from megaloblastic marrow incubated with sickle marrow nucleoprotein (experimental) were compared with those separated from the same marrow incubated without nucleoprotein (control). The radioactivity present in each eluate fraction was determined and expressed as a ratio of counts per minute to optical density at 580 mμ cpm/O.D.). An abnormal hemoglobin component was eluted at a higher pH than the major normal component (*x*). A peak of radioactivity coincides with this abnormal hemoglobin component indicating increased amino acid incorporation and new hemoglobin synthesis.

The eluates containing the abnormal hemoglobin components were concentrated and a peptide fingerprint analysis performed. These were compared to the peptides present in the major normal hemoglobin component derived from the same incubation mixture (Fig. 2, II) as well as those derived from the hemoglobin components present in the control incubation mixture (Fig. 2, peaks I and II). Two peptides were present in an unusual position in the fingerprint of the abnormal hemoglobin component (Fig. 4, *A* and *B*) when compared to the peptide fingerprint of the control hemoglobins. Similar abnormalities were demonstrable in three additional experiments where peptide fingerprint analyses were performed on the abnormal hemoglobin component isolated by CMC chromatography. One of these two peptides appears to be a displacement of a peptide present in the control hemoglobin (Fig. 5, *A*) and the other peptide is not found in the control hemoglobin (Fig. 5, *B*). Thus 28 peptides were present in the fingerprint of the control hemoglobin and 29 peptides in that of the abnormal hemoglobin component. In all instances these two abnormal peptides migrated toward the anode. These differences were demonstrable in all experimental samples of hemoglobin when compared with control samples analyzed simultaneously under identical conditions. The peptide fingerprints were not identical with those obtained from known samples of sickle cell hemoglobin.

No abnormal hemoglobin components were detectable by electrophoretic proce-

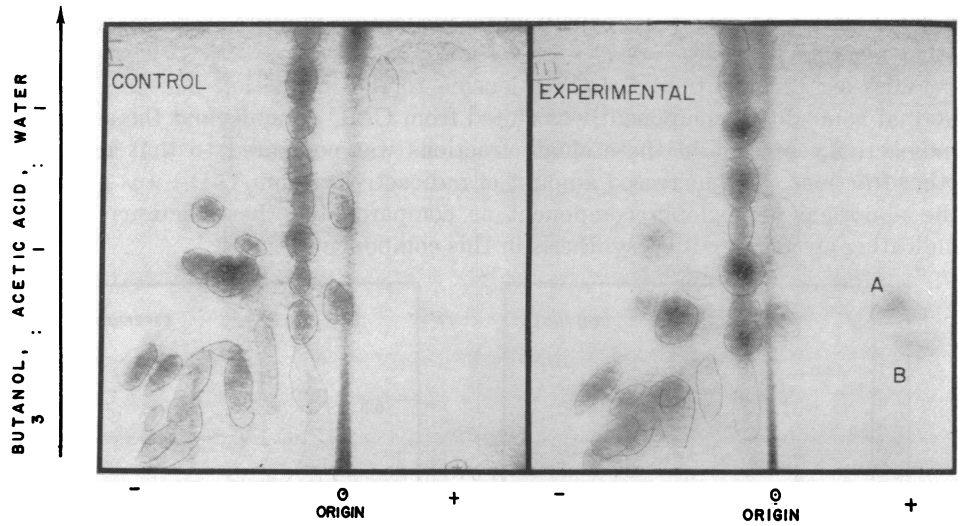

FIG. 4.—Peptide fingerprint analysis of hemoglobin components separated by carboxymethylcellulose column chromatography. The hemoglobins were obtained by concentrating fractions eluted from CMC columns (Fig. 2), digested with trypsin and separated in two dimensions by electrophoresis and ascending chromatography. Peptides derived from the abnormal component isolated from the experihemoglobin (x, Fig. 2) are compared with those obtained from the major normal components (II, Fig. 2) in both the control and experimental hemoglobin samples. Two peptides (A, B) are demonstrable in the abnormal component. No similar peptides were present in a comparable region in other fractions of either the control or experimental hemoglobin.

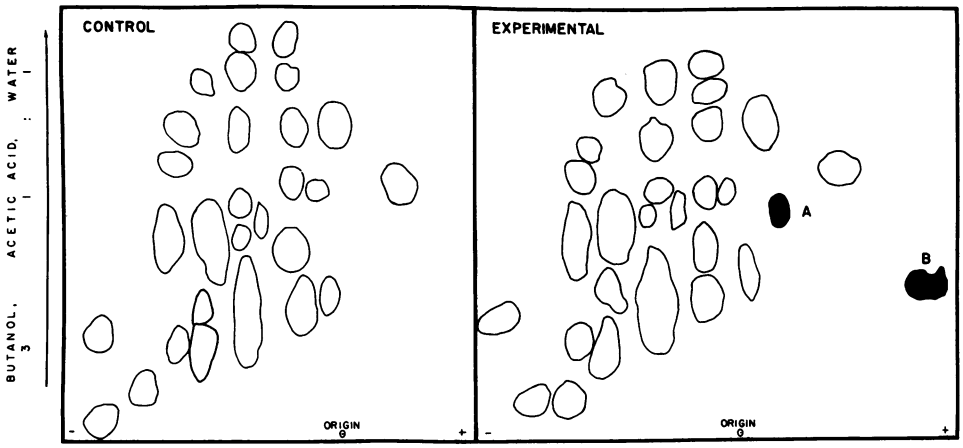

FIG. 5.—Peptide fingerprint analysis of an abnormal hemoglobin component compared with that obtained from normal hemoglobin. Tryptic digestion, electrophoresis and chromatography of the two samples were performed simultaneously under identical conditions. Two peptides (A and B) are present in an unusual position in the fingerprint of the abnormal component. One of these appears to be a displacement of a peptide present in the control hemoglobin (A). No peptide comparable to B is present in the control. There are 29 peptides present in the experimental hemoglobin compared to 28 in the control. Electrophoresis was carried out for 3½ hr, as in Fig. 4, in order to obtain better separation of the peptides.

dures or by CMC column chromatography when megaloblasts were incubated with nucleoproteins derived either from normal marrow or from multiple myeloma marrow cells (Table 1).

Incubation of sickle cell anemia marrow with nucleoproteins isolated from normal

TABLE 1

INCUBATION MIXTURES OF CELLS AND NUCLEOPROTEINS USED TO DETERMINE ALTERED HEMOGLOBIN FORMATION

Incubation Mixture		Altered Hemoglobin Formation
Cells	Nucleoprotein	
Megaloblasts	Sickle marrow	+
Megaloblasts	Normal marrow	0
Megaloblasts	Myeloma marrow	0
Normal reticulocytes	Sickle marrow	+
Sickle marrow	Normal marrow	+
Sickle reticulocytes	Normal marrow	+
Sickle reticulocytes	Normal spleen	0

marrow: Altered hemoglobin formation was also evident in an incubation mixture composed of marrow aspirated from an individual with sickle cell anemia and nucleoprotein derived from normal marrow (Table 1). The altered component was eluted from CMC columns and was present in the hemoglobin solutions derived from the experimental hemoglobin solutions but not in that derived from the control. This hemoglobin component was eluted from the column at a lower pH than the components normally present in sickle hemoglobin, resembling normal hemoglobin in this respect.[23]

Incubation of reticulocytes with nucleoproteins isolated from genetically different marrow: Abnormal globin formation was also demonstrable by CMC column chromatography when peripheral blood containing 20 to 40 per cent reticulocytes was incubated with marrow nucleoprotein isolated from an appropriate source (e.g., from marrow with different genetic hemoglobin) (Table 1). Thus sickle cell peripheral blood incubated with a normal marrow nucleoprotein preparation exhibited an altered hemoglobin component which resembled normal hemoglobin in its elution behavior from CMC columns (Fig. 6). No altered hemoglobin formation

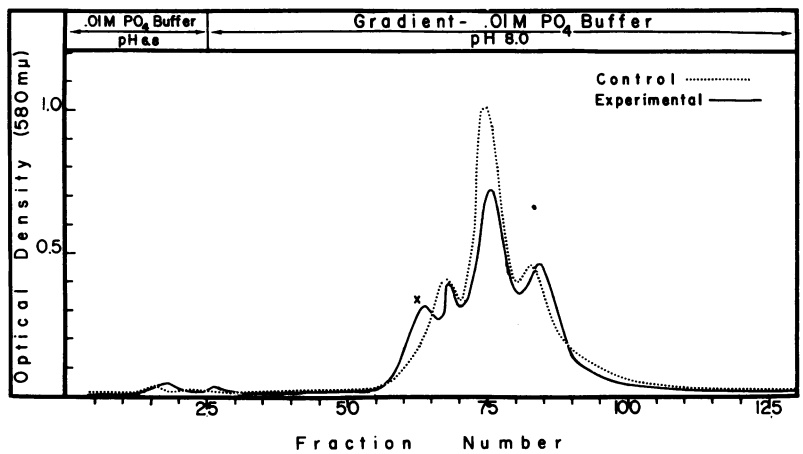

FIG. 6.—Hemoglobin components obtained from incubation mixtures of sickle cell anemia reticulocytes incubated with normal marrow nucleoprotein (experimental), compared to these derived from the same blood incubated without nucleoprotein (control). The hemoglobin components were separated by CMC column chromatography. An abnormal component (x) was present in the hemoglobin derived from the experimental incubation mixture but not present in the control. The abnormal hemoglobin component (x) elutes at a lower pH than the sickle components, behaving like normal hemoglobin in this respect.

was, however, detected when sickle cell reticulocytes were incubated with nucleoprotein derived from normal spleen.

Peripheral blood from a normal individual recovering from blood loss (21 per cent reticulocytes) was incubated with nucleoprotein derived from sickle cell anemia marrow. An abnormal hemoglobin component was again demonstrated by CMC column chromatography. The abnormal hemoglobin was eluted at a higher pH than the major normal component, resembling sickle hemoglobin in this respect.

The effect of enzymatic degradation of nucleoproteins on altered hemoglobin formation: The effect of various degrading enzymes on the activity of the nucleoprotein preparation in the production of altered hemoglobin was evaluated (Table 2). The

TABLE 2

THE EFFECT OF ENZYMATIC DEGRADATION OF NUCLEOPROTEIN ON ALTERED HEMOGLOBIN PRODUCTION IN IMMATURE ERYTHROCYTES

Nucleoprotein modification	Substances present	Altered hemoglobin formation
None	DNA RNA Protein	+
DNA-ase	RNA Nucleosides Nucleotides Protein	+
RNA-ase	DNA Nucleosides Nucleotides Protein	0
DNA-ase RNA-ase	Nucleosides Nucleotides Protein	0
Trypsin	DNA RNA	0

nucleoprotein preparation was incubated with either DNA-ase (5 μg/ml), RNA-ase (5 μg/ml), or trypsin (5 μg/ml) prior to its addition to the incubation mixture. For purposes of comparison, megaloblasts were incubated with the untreated nucleoprotein preparation derived from sickle cell anemia marrow and the hemoglobin components separated by CMC column chromatography. These hemoglobin components were then compared with those obtained when the megaloblasts were incubated with sickle nucleoprotein previously incubated with DNA-ase; with that obtained when the nucleoprotein was incubated with RNA-ase and with that obtained when the nucleoprotein was incubated with trypsin.

Preincubation of the nucleoprotein complex with DNA-ase did not abolish the altered hemoglobin formation (Fig. 7). On the contrary, in three experiments, preincubation of the nucleoprotein with DNA-ase appeared to enhance the formation of the abnormal hemoglobin component. In fact, altered hemoglobin formation was demonstrable in these experiments after incubation of the nucleoprotein complex with DNA-ase even though abnormal hemoglobins were not detected when the marrow cells were incubated with the whole nucleoprotein complex.

Preincubation of the nucleoprotein with RNA-ase consistently resulted in lack of altered hemoglobin formation. A similar loss of ability to induce altered hemoglobin formation occurred when the nucleoprotein was incubated with trypsin (Table 2).

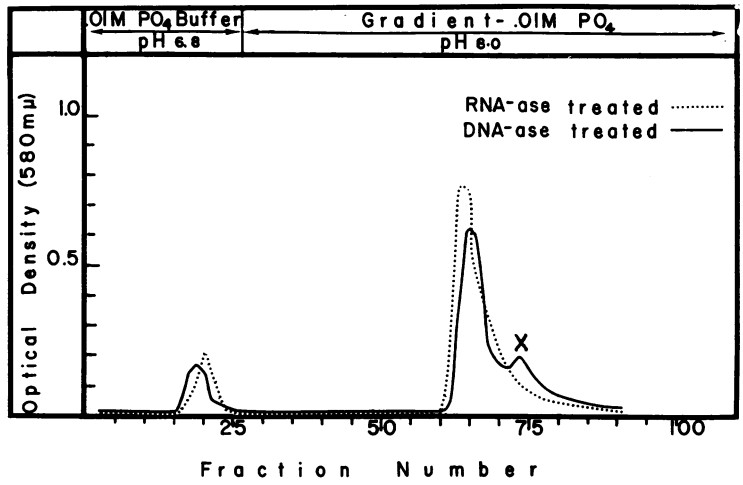

FIG. 7.—The effect of enzymatic degradation of nucleoprotein on the production of abnormal hemoglobin by immature erythrocytes. Sickle marrow nucleoprotein was degraded with either DNA-ase or RNA-ase prior to incubating the material with normal reticulocytes. The abnormal hemoglobin component (x) formed in such incubation mixtures was demonstrated by CMC column chromatography. Pretreatment of the sickle marrow nucleoprotein with DNA-ase did not prevent the formation of an abnormal hemoglobin. Pretreatment of the nucleoprotein with RNA-ase resulted in loss of ability to induce abnormal hemoglobin formation.

Identical results were obtained with other incubation mixtures employing reticulocytes. Thus DNA-ase did not affect abnormal hemoglobin formation when normal reticulocytes were incubated with sickle marrow nucleoproteins. Pretreatment of the nucleoprotein with RNA-ase or trypsin resulted in loss of the ability to induce altered hemoglobin formation. The same results were also obtained when sickle cell anemia reticulocytes were incubated with normal marrow nucleoprotein degraded in the same manner.

Comment.—These data demonstrate that altered hemoglobin synthesis can be induced when immature erythrocytes are incubated with nucleoprotein derived from individuals with genetically different marrow. The altered hemoglobin is demonstrable by electrophoretic techniques, CMC column chromatography, and by peptide fingerprint analysis after tryptic digestion. The increased amino acid incorporation (1-C^{14}-leucine) into the abnormal hemoglobin component (cpm/O.D.) as well as the distinctly different peptide fingerprint analysis indicates that this component is due to newly synthesized hemoglobin rather than to any alteration of preexisting hemoglobin.

The altered globin production is dependent upon the presence of intact RNA and protein in the nucleoprotein preparation. This is indicated by the inactivation of the preparation when degraded by either RNA-ase or trypsin. The identity of the ribonucleoprotein responsible for the observed effects is being investigated by fractionation procedures. In all probability the RNA-protein present in the nucleoproteins employed in these studies represents only a small fraction of that present in the cell. The initial nucleoprotein isolation procedures were designed primarily to study the effect of DNA and it is conceivable that an active ribo-

nucleoprotein fraction, closely associated with DNA, was fortuitously isolated by the procedures employed. The possibility that these preparations contain either microsomal RNA or messenger RNA is not excluded. The activity of other cellular fractions of ribonucleoprotein also remains to be determined.

The role of protein present in these nucleoprotein preparations in relation to the altered globin production by immature erythrocytes is not completely elucidated. Although incubation of the nucleoprotein with trypsin resulted in a loss of the ability to induce hemoglobin changes, the presence of protein alone following enzymatic degradation of the other components was not sufficient to induce altered hemoglobin formation. The possibility exists that the protein stabilizes the RNA and that tryptic digestion affects the RNA in some deleterious manner. It is also possible that the protein fraction does have a definitive role in the new hemoglobin synthesis in conjunction with RNA, possibly that of containing information. Further experiments attempting to elucidate the role of protein in the altered globin synthesis are in progress.

DNA apparently does not contribute to altered protein production in this *in vitro* system since pretreatment with DNA-ase did not result in any loss of the ability to induce the hemoglobin change. A DNA effect might not be evident in the cell systems used in this study since only one generation of cells was involved. However, a DNA effect on information transfer might be demonstrable in cell cultures where several generations could be studied. The apparent enhancement of altered hemoglobin synthesis following incubation of the nucleoprotein with DNA-ase suggests that DNA may compete with the RNA-protein for acceptor sites or possibly complex the RNA-protein so as to render it less effective.

Specific information encoded in the nucleoprotein is apparently required for altered protein synthesis to occur. It would appear that the information is present in nucleoprotein isolated form marrow but not in that isolated from spleen. Conceivably, however, the information is present in the splenic nucleoprotein but not in sufficient quantity to be effective in the systems studied. The lack of altered hemoglobin formation when normal immature erythrocytes are incubated with normal marrow nucleoprotein indicates that the effects observed are not due to a nonspecific effect of nucleoproteins. This is further substantiated by the kinetic studies in which 18 to 24 hr were required for the abnormal component to become apparent and in which no abnormalities were detected at zero time.

Altered hemoglobin formation was demonstrable in all types of immature erythrocytes tested provided that they were capable of synthesizing hemoglobin and were incubated with marrow nucleoproteins isolated from an appropriate genetic source. The reproducibility of the observed effects by the techniques employed was approximately 65 per cent, an alteration in protein synthesis being demonstrable in 16 or 24 experiments. The degree of cellular immaturity was not critical, nor was the presence of a nucleus essential for the observed effects since altered hemoglobin synthesis was as effective in reticulocytes as it was in magaloblasts. Reticulocytes are known to be capable of protein synthesis[24-26] and are therefore very suitable for this type of study. The absence of a nucleus does not necessarily rule out the possible participation of DNA in the altered synthesis. In fact, the presence of DNA within reticulocytes has been demonstrated.[27]

The identity of the altered protein formed when sickle marrow nucleoprotein

is incubated with immature erythrocytes has not been definitely established. The altered hemoglobin component resembles sickle hemoglobin in its electrophoretic behavior and in its elution from CMC columns. The peptide fingerprints are not, however, identical with those obtained from known samples of sickle hemoglobin. Analyses of the amino acid sequences in the abnormal peptides are not in progress and may help characterize the nature of the altered protein.

Although it is not possible at this time to categorize completely the new hemoglobin formed in these incubation mixtures, the possibility that a directed synthesis of globin by ribonucleoprotein has been obtained is suggested by the elution patterns obtained from CMC columns. Thus incubation of megaloblasts with sickle marrow ribonucleoprotein resulted in the formation of an altered hemoglobin component which resembled sickle hemoglobin in its elution from CMC columns. Conversely, incubation of sickle cell reticulocytes with normal marrow ribonucleoprotein resulted in the formation of a hemoglobin component which resembled normal hemoglobin in its elution from CMC columns.

The incorporation of P^{32}-labeled nucleoprotein into marrow cells,[28] as well as into other mammalian cells, has been demonstrated.[29-31] Nucleoproteins entering the cell may contain only fragments of information which are then only partially effective in transferring information within a highly organized cell system. It appears, however, that the nucleoprotein entering the cell is surprisingly efficient in causing abnormal globin formation. Calculations based on the number of O.D. units of hemoglobin eluted from CMC columns indicate that the abnormal component constitutes as much as 3 per cent of the total. It is therefore possible that replication of the RNA-protein occurs within the cell.

These findings indicate that an alteration of information transfer within immature erythrocytes can be effected by a ribonucleoprotein. The altered peptide formation in the newly synthesized abnormal hemoglobin component may be attributed to the successful introduction of an RNA template containing new information which then competes with or interferes with the preexisting information system.

Summary.—Altered globin production can be induced in human immature erythrocytes when incubated with nucleoprotein preparations isolated from genetically different marrow. The altered globin production is dependent upon the presence of both intact RNA and protein in the nucleoprotein preparation. DNA is not required for this effect and its degradation in some experiments enhanced the RNA-protein effect. The information necessary for the abnormal hemoglobin formation is present in marrow nucleoprotein but not in that derived from spleen, although this may be a quantitative rather than a qualitative difference. Abnormal hemoglobin production was observed in all immature erythrocytes capable of synthesizing globin provided they were incubated with ribonucleoproteins isolated from a genetically different marrow.

The alteration of protein synthesis in immature erythrocytes indicates that altered information transfer within human cells can be effected, possibly by incorporating an RNA template containing new information which successfully competes with or interferes with preexisting information systems.

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