strated and the association constants measured (*6*). Both actinomycin and acridine orange binding to DNA are inhibited by divalent cations (*7*). Unlike the bonds between acridine and DNA, those between actinomycin and DNA are thought to be of the hydrogen bond type (*8*).

The details of acridine orange–DNA binding have been described by Bradley and Wolf (*9*). They have characterized two types of binding of acridines to DNA. Type I, which is common to all acridines, is the intercalation model based on the x-ray crystallography studies of Lerman (*10*). This bond has strong electrostatic forces of the order of 6 to 10 kcal/mole and is relatively resistant to divalent cations. The plane of the bound acridine molecules is approximately perpendicular to the DNA axis. Type I binding is favored by double-stranded DNA and results in untwisting and lengthening of the helix. Type II binding is a predominant characteristic of only a few dyes of which acridine orange is usually cited as the chief example. The binding site is external to the helix. Acridine molecules are thought to attach to the helix with the positively charged ring nitrogen close to the negatively charged deoxyribose phosphate groups. The bonding forces are weaker, favored by a disordered or single-stranded DNA and readily affected by divalent cations. Type II binding results in stacking of aggregates of acridine molecules whose number is limited by the number of charged residues. Since all the acridine derivatives tested here exhibit type I binding (intercalation) and only acridine orange is effective, it is clear that intercalation per se is not sufficient to potentiate actinomycin uptake. Type II binding, however, which is a particular feature of acridine orange, may be a necessary prerequisite for this effect. Both type I and type II binding of acridine have in common the fact that the positively charged ring nitrogen is in apposition to the negatively charged oxygen atoms of the backbone phosphate groups. The negative charges of the phosphate groups tend to repel each other in native DNA and unless opposed by counterions tend to unwind the DNA molecule (*11*). The interaction of aminoacridine and DNA appears to favor areas of adjoining purine base pairs rather than pyrimidine pairs (*12*), so that the apposition to negative phosphate groups is not random. The purine base guanine was selected by Reich (*4*) as the probable site of actinomycin attachment to DNA. It is possible that the steric alterations produced by acridine orange in this location could shift additional guanine residues to the precise configuration necessary for actinomycin binding.

Although actinomycin D is presently used in the treatment of Wilms' tumor, Ewing's tumor, rhabdomyosarcoma, and a number of anaplastic sarcomas (*13*), it is of little therapeutic value in the treatment of leukemia, lymphoma, and other commonly occurring neoplasms. The enhancement of actinomycin uptake by acridine orange may broaden the spectrum and efficacy of this antineoplastic agent.

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## Adult Hemoglobin Synthesis by Reticulocytes from the Human Fetus at Midtrimester

Abstract. *The synthesis of adult-type hemoglobin was measured in small samples of peripheral blood cells from 9- to 18-week human fetuses. Hemoglobin indistinguishable from hemoglobin A was identified by ion-exchange chromatography, electrophoresis at pH 8.6, tryptic peptide analysis, and the insensitivity of its synthesis to the action of O-methylthreonine. Synthesis of hemoglobin A accounted for 8 to 14 percent of total hemoglobin synthesis and was demonstrated in as little as 10 microliters of fetal blood. These studies provide sensitive methods for the detection of β chain types in hemoglobin synthesized by the human fetus at midtrimester. If methods to obtain small quantities of fetal blood at midtrimester become available, these techniques should be applicable to the antenatal detection of sickle cell anemia and related hemoglobinopathies.*

Hemoglobin F ($\alpha_2 \gamma_2$) comprises 70 to 90 percent of human hemoglobin at birth (*1*), while hemoglobin A ($\alpha_2 \beta_2$) is the predominant hemoglobin in the adult. Differences in the amino acid sequences of the $\gamma$ chain of hemoglobin F and the $\beta$ chain of hemoglobin A have been elucidated (*2*). Since mutations of the $\beta$ chain are responsible for most hemoglobinopathies observed in man, knowledge of $\beta$ chain production in the early fetus is critical for the antenatal detection of these conditions. Little information is available regarding the synthesis of adult hemoglobin during this period of human development.

In sheep (*3*) and mice (*4*) adult-type hemoglobin appears early in fetal development. In the human, data suggesting the presence of hemoglobin A in the maturing fetus (25 to 70 mm crown-to-rump) has been reported (*5*). Walker and Turnbull used alkali denaturation to determine the proportion of hemoglobin other than hemoglobin F in the human fetus aged 11 to 43 weeks (*6*). No alkali-denaturable hemoglobin was detected in blood samples from four fetuses aged 11 to 12 weeks. At week 13, 1 to 2 percent of the total hemoglobin was alkali-sensitive, and presumably this hemoglobin was other than hemoglobin F. The proportion of alkali-sensitive hemoglobin increased to 10 percent by weeks

22 to 24, remained at 10 percent until week 35, and then rose to about 20 percent at term.

We have ascertained the amount of hemoglobin A in the human fetus, and studied the incorporation of radioactive amino acids into newly synthesized hemoglobin A by fetal peripheral blood cells. The isoleucine analog, L-*O*-methylthreonine (OMT) was used to inhibit selectively the synthesis of hemoglobin F. This analog inhibits the incorporation of isoleucine into protein by producing a deficiency of isoleucyl-transfer RNA (*7*). since $\gamma$ chains contain four isoleucine residues while the $\alpha$ and $\beta$ chains contain no isoleucine, OMT inhibits the synthesis of hemoglobin F without effect on the synthesis of hemoglobin A in reticulocytes of the newborn (*8*). We have found that hemoglobin A is synthesized by reticulocytes of the midtrimester fetus in quantities sufficient to allow its detection in 10 $\mu$l of fetal blood.

Fetal umbilical cord blood, free from contamination with maternal blood, was obtained from 9- to 18-week fetuses immediately after delivery by elective hysterectomy or hysterotomy. These samples were collected in ice-cooled glass tubes containing heparin. At this stage of gestation a high proportion of erythrocytes were immature (that is, 80 percent reticulocytes and 1 percent nucleated red blood cells), as was expected (*9*).

Red cells were washed twice with low magnesium saline (*10*) and were incubated at 37°C for 2.5 or 3.0 hours (*7*). The incubation medium contained all rate-limiting amino acids required for hemoglobin synthesis (except those added in radioactive form), ferrous ammonium sulfate, pooled human transferrin, glucose, and tris[tris(hydroxymethyl)aminomethane]-HCl buffer. After the cells were suspended in the incubation medium, radioactive amino acids were added as follows: 9 $\mu$c of [$^{35}$S]methionine (12 c/mmole) per 0.1 ml of packed cells or 5 $\mu$c each of [$^{3}$H]valine (16.4 c/mmole) and [$^{3}$H]leucine (58 c/mmole) per 1.0 ml of packed cells. When OMT was used at a final concentration of 25 m*M*, it was added 5 minutes before the radioactive amino acids were added. The incubating reactions were terminated by cooling in ice, and cells were collected by centrifugation, washed twice with low magnesium saline, and lysed in four volumes of 5 m*M* $MgCl_2$. Hemoglobin solutions were clarified by centrifugation at 16,000*g* for 10 minutes, and dialyzed against distilled water. The hemoglobin was then saturated with carbon monoxide and stored frozen for further analysis.

In a typical experiment, the mixture of hemoglobins labeled with [$^{35}$S]-methionine had a specific activity of approximately 10,000 to 20,000 count/min per milligram. This corresponded to approximately 10,000 count/min isolated from an amount of peripheral fetal blood containing 5 $\mu$l of packed red cells.

Hemoglobin isolated from fetal blood cells and containing newly synthesized radioactive protein, was combined with nonradioactive "carrier," adult hemoglobins A and S, and analyzed by ion-exchange chromatography in sodium phosphate buffer at *p*H 6.85. A modification of the method of Clegg and Schroeder (*11*) was used with small columns (0.5 by 7 cm) of cation exchange resin (Bio-Rex 70) and a stepwise gradient of ionic strength for elution. Three major hemoglobin peaks were observed (Fig. 1). The first peak, eluted in the cold, was well separated from the two peaks eluted after the column was warmed to room temperature (25°C). Immediately after elution of the second peak, the third hemoglobin peak was eluted with a stepwise increase in the strength of the eluting buffer from 0.055*M* $Na^+$ to 0.11*M* $Na^+$. Electrophoresis of protein from the peaks (Fig. 2A, channels 3 to 5) in cellulose acetate gels at *p*H 8.6 indicated that the first peak contained hemoglobin F, the second, hemoglobin A, and the third, hemoglobin S. In separate experiments, when hemoglobins $A_2$ and C were added as "carriers" they could be eluted from the column after hemoglobin S, provided that the strength of the eluting phosphate buffer was further increased to 0.22*M* $Na^+$.

In the samples studied, peaks of

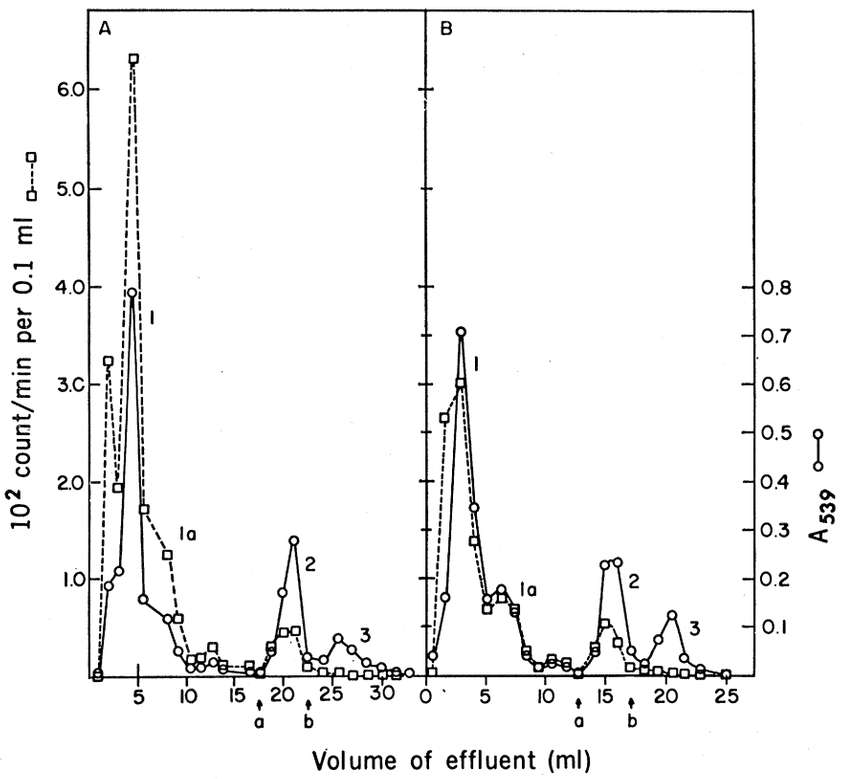


Fig. 1. Chromatography of hemoglobins on Bio-Rex 70. (A) Analysis of hemoglobin synthesized in the absence of *O*-methylthreonine. (B) Analysis of hemoglobin synthesized in the presence of *O*-methylthreonine. Samples containing 3 mg of radioactive hemoglobins from a 16-week fetus and 1.3 mg of a mixture of "carrier" hemoglobins A and S were applied in 0.16 ml of developer No. 5 (*11*). The arrows indicate changes in elution conditions: *a*, column is warmed from 4°C to 25°C; *b*, change from eluting buffer of 0.055*M* $Na^+$ to 0.11*M* $Na^+$. Open circles, absorbancy (*A*) at 539 nm (1-cm path); open squares, counts per minute above background in 0.1-ml portions of effluent. Peak 1, hemoglobin F; peak 2, hemoglobin A; peak 3, hemoglobin S (experiment I-6, a and b, Table 2).

radioactivity were eluted with hemoglobin F and hemoglobin A but not with hemoglobin S (Fig. 1). Material with a relatively high specific activity was eluted just before the main peak of hemoglobin F. The exact nature of this material is unknown. Protein in the shoulder of the hemoglobin F peak (Fig. 1A) had a specific activity comparable to that of the hemoglobin in the major portion of the peak. This material could not be distinguished by electrophoresis at *p*H 8.6 from hemoglobin F in peak 1 (Fig. 1).

The effect of OMT on hemoglobin synthesis in these cells was demonstrated by column chromatography (Fig. 1). The specific activity of hemoglobin F synthesized in the presence of OMT (Fig. 1B) was about 50 percent of the specific activity of hemoglobin F observed in the absence of OMT (Fig. 1A). In contrast, the radioactivity in the peak of hemoglobin A (peak 2, Fig. 1, A and B) was essentially unaffected by OMT (Table 2).

The radioactive protein eluted with hemoglobin A was analyzed further by electrophoresis at *p*H 8.6 (Fig. 2B). The radioactivity was confined to the position of "carrier" hemoglobin A. Neither other protein nor radioactivity was detected in the gel at positions where hemoglobins $A_2$, C, S, or F would migrate.

In two experiments, newly synthesized hemoglobin from fetal red cells, labeled with [$^3$H]leucine and [$^3$H]valine, was subjected to chomatography in the absence of added "carrier" hemoglobins (Fig. 3). Radioactive hemoglobin F was eluted in the first peak. A small amount of radioactively labeled hemoglobin, accounting for 4 to 5 percent of the total hemoglobin detected by spectrophotometry, was eluted in the second peak. Hemoglobin from the first peak had the electrophoretic mobility at *p*H 8.6 of hemoglobin F; the mobility of hemoglobin from the second peak was identical to that of hemoglobin A (Fig. 2A, channel 2).

The identity of these radioactive hemoglobins was further confirmed by tryptic peptide analysis (*12*), with the use of high-voltage electrophoresis at *p*H 6.4 and *p*H 3.5 in pyridine–acetic acid buffers, and descending paper chromatography (Table 1). The pattern obtained for hemoglobin from peak 2 (Fig. 3) was that expected for hemoglobin A, and was clearly distinct from that of hemoglobin F isolated from the same fetal blood sample. The difference between the two peptide patterns was supported with the use of the iodoplatinate reagent (*13*) to detect the large methionine-containing peptide, $\beta$ TpV (*14*) of hemoglobin A. This negatively charged peptide was located in its expected position after electrophoresis at *p*H 6.4. No methionine-containing peptide was found in this position in digests of hemoglobin F. This result is expected since no tryptic peptide of hemoglobin F with a negative charge at *p*H 6.4 contains methionine. The distinguishing peptides, $\gamma$ TpIX and $\beta$ TpIX, as well as the chymotryptic peptide composed of residues 141 to 144 of the $\beta$ chain (*15*), were found, by ninhydrin reagent, in appropriate locations in the peptide patterns of hemoglobin F and hemoglobin A, respectively.

The data given in Table 1 indicate that all peptides isolated from hemoglobin A, fragments of both $\alpha$ and $\beta$ chains, had specific activities four to ten times greater than those of comparable peptides isolated from hemoglobin F. The differences in specific activities were not due to unequal recovery of the peptides from hemoglobins A and F (*16*). Thus, the 25-fold difference in pool size of hemoglobin F and hemoglobin A, in addition to the marked inhibition of $\gamma$-chain synthesis by OMT, accounts for the disparity in specific activities of $\beta$ and $\gamma$ chains. In the presence of OMT, a large excess of free $\alpha$ chains is synthesized, and these equilibrate slowly with the $\alpha$ chains of hemoglobins A and F (*8*). Therefore, the differences in specific activities of $\alpha$ chains from hemoglobins A and F reflect the availability of newly synthesized $\beta$ and $\gamma$ chains to form these hemoglobin molecules.

Chromatography analysis was used to compare the synthesis of hemoglobin A to that of hemoglobin F (Table 2). The percentage of hemoglobin A was based on the amount of radioactivity found in the peak of hemoglobin A, relative to the total radioactivity recovered in the peaks of both hemoglobin A and hemoglobin F. Correction was then made for the number of labeled

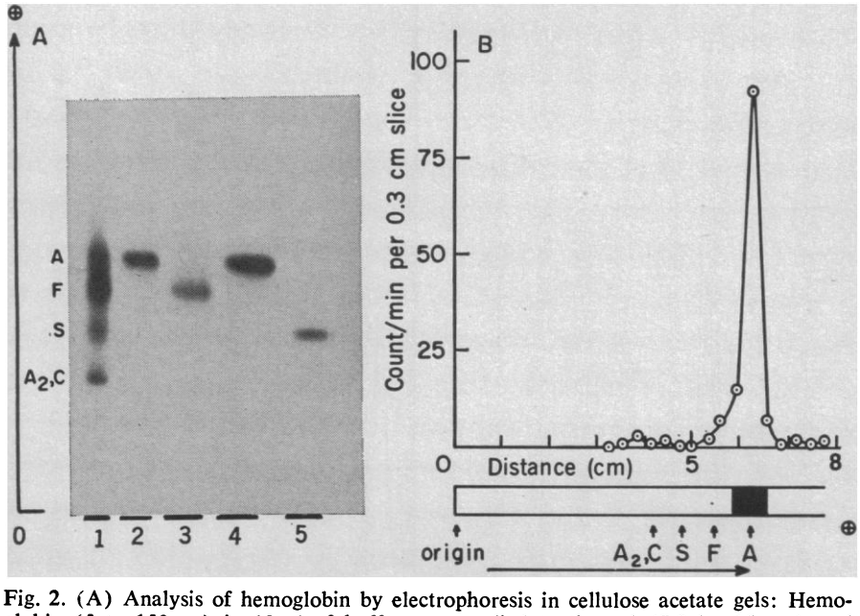


Fig. 2. (A) Analysis of hemoglobin by electrophoresis in cellulose acetate gels: Hemoglobin (5 to 150 $\mu$g) in 10 $\mu$l of buffer was applied to the gel (7.8 by 15 cm) and subjected to electrophoresis at *p*H 8.6 for 4 hours at 4°C (constant 4 ma, ~ 400 volt). The buffer, modified from Smithies (*18*), contained 0.18*M* tris, 0.002*M* disodium ethylenediaminetetraacetate, and 0.10*M* boric acid at *p*H 8.6. Protein was detected with nigrosine 0.5 percent in a mixture of methanol, acetic acid, water (9 : 2 : 9 by volume). Channel 1, marker sample containing hemoglobins A, F, S, C, and $A_2$ as indicated; channel 2, hemoglobin isolated by ion-exchange chromatography in the absence of added "carrier" hemoglobin, obtained from peak 2, Fig. 3; channels 3 to 5, analysis of hemoglobins isolated in the presence of added "carrier" hemoglobins A and S as in Fig. 1; channel 3, hemoglobin F from peak 1, Fig. 1; channel 4, hemoglobin A from peak 2, Fig. 1; channel 5, hemoglobin S from peak 3, Fig. 1. (B) Radioactivity in slices (1 by 0.3 cm) of gel from channel 4. Gel slices were dissolved in 1 ml of 80 percent acetic acid, and nigrosine was bleached with one drop of 1 percent calcium hypochlorite; 10 ml of water-miscible scintillation medium (*19*) was added for measurement of radioactivity. Efficiency of $^{35}$S analysis was approximately 60 percent. Beneath the radioactivity profile is a representation of channel 4, with arrows denoting positions of marker hemoglobin samples as demonstrated in channel 1.

amino acids in each hemoglobin molecule: for example, six methionine residues in hemoglobin A and eight methionine residues in hemoglobin F. It was possible to obtain these data from amounts of fetal blood ranging from 10 to 700 $\mu$l and containing from 2.5 to 100 $\mu$l of packed red cells. In 45- to 130-mm (crown-rump) fetuses, the amount of hemoglobin A synthesis was 8 to 14 percent of total hemoglobin synthesis, a minimum estimate, because the early portion of the hemoglobin F peak contains radioactivity not incorporated in hemoglobin. There was no evidence that hemoglobin A eluted from the ion-exchange column was contaminated as judged by subsequent electrophoresis.

We conclude that in the fetus at midtrimester a hemoglobin identical to hemoglobin A, with respect to its tryptic peptide pattern and the insensitivity of its synthesis to the presence of OMT, is produced. Moreover, column chromatography and gel electrophoresis demonstrated the synthesis of hemoglobin A in red cells from the 10-week human fetus. Our results (i) confirm and extend the observations of Walker and Turnbull (*6*), which were based on alkali-denaturation studies, and (ii) show that, while the proportion of hemoglobin A present in the 16-week fetus (determined spectrophotometrically) was 4 to 5 percent, the proportion of newly synthesized hemoglobin A was nearly double, that is, 10 percent. These findings are in close agreement with the alkali-sensitive fraction of hemoglobin (10 percent) which obtains in fetuses from 19 to 34 weeks (*6*). However, the levels of synthesis of hemoglobins F and A observed in peripheral blood cells in vitro may not reflect the actual proportions of synthesis of these hemoglobins by cells at earlier stages of erythroid maturation in vivo.

The techniques used were sufficiently sensitive and specific to detect small amounts of $\beta^s$ chain synthesis and to differentiate the AS heterozygote from the SS homozygous fetus. Since only newly synthesized hemoglobins are considered in this analysis, as much as 50 percent contamination of the blood sample by maternal erythrocytes (maternal reticulocyte count equal to or less than 2 percent) should not affect the interpretation of the data. No hemoglobin S was synthesized in any of the fetal blood samples studied. Thus, we believe that the $\beta^s$ allele was not present in the four fetuses studied.

A small number of fetal erythro-

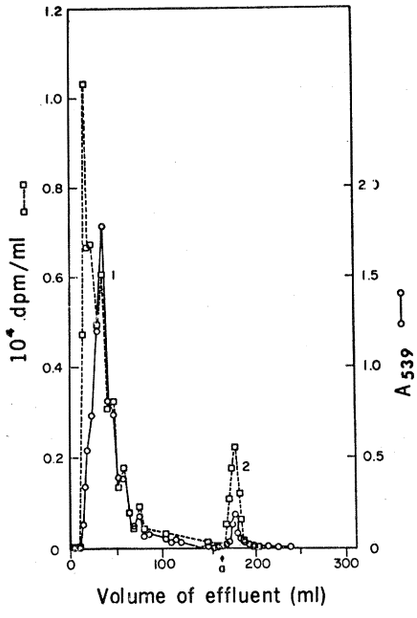


Fig. 3. Chromatography of hemoglobins from fetal blood in the absence of added "carrier" hemoglobins: approximately 60 mg of hemoglobin in 3 ml of eluting buffer were applied to the column (1 by 15 cm). Elution was performed at 4°C with a flow rate of 10 ml per hour. When the extinction of effluent fell to zero, the column was warmed (arrow *a*) to approximately 25°C, and elution was continued with the same developer until the second peak was eluted. Open circles, absorbancy at 539 nm (1-cm light path); open squares, radioactivity above background in 0.1-ml portions of effluent (dpm, corrected for self-absorption). (Experiment I-6c, Table 2.) In this experiment and others, approximately 70 percent of the radioactivity applied to the ion-exchange column was recovered.

Table 1. Analysis of radioactive peptides from hemoglobins synthesized in the presence of *O*-methylthreonine. Peptide analysis was performed on trypsin digests of 6 to 7 mg of globin. Peptides were eluted from filter paper with 0.1*N* HCl for measurements of radioactivity of [$^3$H]valine and [$^3$H]leucine. The radioactivity reported is corrected for self-absorption of the samples. Efficiency of $^3$H detection was 20 percent. Nonninhydrin reactive areas of paper in the vicinity of each peptide showed the following radioactivity in disintegrations per minute (dpm) over background: $\beta$ TpV, 33; $\beta$ peptide 141–144, 20; $\beta$ TpIX, 32; $\gamma$ and $\alpha$ peptides of hemoglobin F, $<10$ dpm (*14*). Peptides uniquely characteristic of the $\beta$ chains of hemoglobin A are denoted by an asterisk.

| Hemoglobin A | | | | Hemoglobin F | | | |
|---|---|---|---|---|---|---|---|
| Peptide | dpm/ 6.8 mg digest | Content of | | Peptide | dpm/ 6.8 mg digest | Content of | |
| | | Val | Leu | | | Val | Leu |
| $\alpha$ TpV | 289 | 0 | 1 | | | | |
| $\alpha$ TpVI | 528 | 1 | 1 | $\alpha$ TpVI | 128 | 1 | 1 |
| $\alpha$ TpIX | 1586 | 3 | 4 | $\alpha$ TpIX | 200 | 3 | 4 |
| $\beta$ TpII | 324 | 1 | 1 | $\gamma$ TpII | 34 | 1 | 1 |
| *$\beta$ TpV | 134 | 1 | 1 | | | | |
| $\beta$ TpVI | 476 | 1 | 0 | $\gamma$ TpVI | 45 | 1 | 0 |
| $\alpha$ TpVIII & $\beta$ TpVIII | 4 | 0 | 0 | $\alpha$ TpVIII & $\gamma$ TpVIII | 0 | 0 | 0 |
| *$\beta$ TpIX | 696 | 1 | 4 | $\gamma$ TpIX | 36 | 1 | 2 |
| *$\beta$ Residues 141–144 | 93 | 0 | 1 | | | | |

Table 2. Hemoglobin A synthesis in fetal reticulocytes. Radioactively labeled hemoglobins synthesized by fetal reticulocytes were combined with nonradioactive adult "carrier" hemoglobins A and S (HbA, HbS). After ion-exchange chromatography, radioactivity eluted in the peaks of hemoglobins A and F were measured. The percentage of hemoglobin A synthesized when [$^{35}$S]methionine was used:

$$100 \times \frac{X_A}{6} \Big/ \left(\frac{X_A}{6} + \frac{X_F}{8}\right)$$

where $X_A$ is the total radioactivity of HbA, and $X_F$ is the total radioactivity of hemoglobin F. A similar correction was made in this calculation when [$^3$H]valine and [$^3$H]leucine were added. The activity in the shoulder of the hemoglobin F peak (Fig. 1A) was combined with the activity in the main hemoglobin F peak for purposes of these calculations. In one experiment (I-6), several estimates of hemoglobin A synthesis were made under different conditions.

| Experiment | Fetal age (weeks) | Crown-rump length (mm) | OMT (m*M*) | Radioactivity in hemoglobin A | Radioactive label |
|---|---|---|---|---|---|
| I-5 | 7 to 11 | 45 | 0 | 8 | [$^{35}$S]Met |
| I-8 | 9½ | 58 | 0 | 8 | [$^{35}$S]Met |
| I-3 | 11 | 95 | 0 | 14 | [$^{35}$S]Met |
| I-6a | 16 | 130 | 0 | 10 | [$^{35}$S]Met |
| I-6b | 16 | 130 | 25 | 18 | [$^{35}$S]Met |
| I-6c | 16 | 130 | 25 | 15 | [$^3$H]Val and [$^3$H]Leu |
| I-6d | 16 | 130 | 25 | 14 | [$^3$H]Val and [$^3$H]Leu |

cytes are often found in amniotic fluid obtained from hysterotomy samples between the 16th and 20th weeks of pregnancy (*17*). If sufficient numbers of fetal erythrocytes are also present in routine amniotic fluid samples obtained by transabdominal amniocentesis during this period, these techniques should be applicable to the antenatal detection and management of hemoglobinopathies such as sickle cell anemia.

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14. Tryptic peptides are numbered according to the distances of their lysyl or arginyl residue from the $NH_2$ terminus of the unhydrolyzed chain.
15. The term chymotryptic peptide is used because the trypsin preparation has chymotryptic-like activity in cleaving the $\beta$ chain at asparagine residue 140.
16. After digestion of A and F globins, the radioactive peptides solubilized by trypsin were analyzed. The expected radioactivity in the peptides isolated was calculated from the ratio of the number of leucine and valine residues in those specific peptides to the total number of those residues solubilized by trypsin. By this method, the recovery of radioactivity in peptides of hemoglobins A and F was very similar: 20 to 25 percent of the calculated value.
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## Polymorphism of Human Constitutive Heterochromatin

Abstract. *Genetic polymorphism has been demonstrated in man for many characteristics including blood groups, serum proteins, tissue enzymes, and hemoglobins. A class of chromosomal polymorphism involving constitutive heterochromatin has now been found. Through the use of a special technique that permits visualization of heterochromatin, seven heterochromatin variants have been found among four individuals. These results suggest a very high frequency of variability of heterochromatin in the population.*

Arrighi and Hsu (*1*) have described a method by which constitutive heterochromatin can be identified in human chromosomes. The technique, first used with mouse chromosomes (*2*), involves an alkali treatment of the cells in situ and subsequent incubation in a salt solution. This procedure is believed to denature the DNA and then allow partial annealing of the DNA strands during incubation. When the slides are then treated with Giemsa stain, a darkly stained area is produced at the centromere of every chromosome. In addition, very prominent blocks of heterochromatin are stained in the secondary constriction regions of chromsomes No. 1, 9, and 16. The Y chromosome is unique in that it has a large heterochromatin block that covers the distal half of the long arm. We used the procedure of Arrighi and Hsu for the original purpose of differentiating between morphologically similar chromosomes. However, a high frequency of variation was found among individuals, results which suggested a new class of polymorphism in humans.

Slides were made from cultures of human lymphocytes prepared according to the method of Moorhead *et al.* (*3*). Basic protein (histones) was removed by treating cytological preparations with 0.2*N* HCl for 15 minutes. The slides were treated with 0.07*N* NaOH for 1 minute and then incubated in a saline-citrate solution (*4*) at 65°C for 24 hours. The slides were finally stained in buffered Giemsa (*5*) for 15 minutes.

Analyses of 25 cells from each of five individuals revealed some differences among chromosome pairs in groups D, E, and G; however, these differences were often so poorly defined that it was difficult to consistently classify specific pairs. No clear-cut differentiation could be made among the pairs of groups B, C, and F (*6*).

An unexpected result was the discovery that a high frequency of heterochromatin polymorphism occurs within homologs. Only one of five individuals studied had a "normal" heterochromatin pattern in all 46 chromosomes. In the remaining four subjects, we observed seven variant patterns involving chromosomes No. 1, 9, and 16 as well as groups D, F, and G. These seven variants are shown in Fig. 1.

One individual had a chromosome No. 9 (Fig. 1b) with a block of heterochromatin at least twice as long as that normally seen for pair No. 9. The heterochromatin region of its homolog, readily identifiable with this technique, was normal in size. A second individual had two different variants, one chromosome in group D with a heterochromatin block larger than usual (Fig. 1d) and a group G chromosome in which no satellites or stalks were visible in any of the cells examined (Fig. 1f, lower row). Though the short arms do not usually appear darkly stained with this procedure, the treatment tends to loosen the compaction of the stalks and satellites of the acrocentric chromosomes. Thus, greater detail of the short arm morphology is revealed, and a loss of chromatin from this region is more readily apparent.

Each of the remaining two individuals also had two different variants. One individual had a chromosome No. 1 with an unusually long block of heterochromatin (Fig. 1a) and a chromosome No. 16 in which the heterochromatic region was decreased to approximately one-third its normal size (Fig. 1c). The last person had two less obvious though equally consistent deviations, one F chromosome that had an exaggerated amount of centromeric heterochromatin extending into both arms (Fig. 1e) and a G chromosome in which very prominent satellites were unusually dark (Fig. 1f, upper row). Neither of these two patterns is normally seen in groups F and G.