stranded part, and the label is lost during a chase, with the simultaneous appearance of labeled 27*S* RNA. After at 15-minute labeling period most (60 to 80 percent) of the 16*S* RNA is ribonuclease-sensitive.

The minimum time for labeling free 27*S* RNA molecules, which is probably about 0.5 minute in our system, would represent the time for synthesis of the most nearly finished molecule and its displacement by the next in line (the first and longest tail belongs to a completed molecule which will be displaced unlabeled). The RNA of the infecting virus appears to be absent in mature progeny (*16*). In order to reconcile this finding with our model (Fig. 5), in which the infecting strand forms a duplex and is then displaced (*13*), it must be assumed that the strands produced early in infection are less likely to be built into virus particles than those produced later.

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## Amino Acids: Incorporation into α- and β-Chains of Hemoglobin by Normal and Thalassemic Reticulocytes

Abstract. *After incubation of reticulocytes with radioactive amino acid, the specific activity of the β-chain of globin from the blood of patients with thalassemia was consistently lower than that of the α-chain. In subjects without this disorder, the specific activity of the β-chain was always equal to or higher than that of the α-chain.*

Study of the molecular basis of thalassemia is of considerable interest since the disease is the prototype of a genetically controlled anomaly in which there is a decreased production of a structurally normal protein. As such, this defect may provide a means of investigating some aspects of the control of the rate of protein synthesis in a mammalian system (*1, 2*).

We have recently been engaged in the measurement of the rate of uptake of isotopic amino acids into the α- and β-chains of rabbit hemoglobin synthesized in vitro by reticulocytes. After brief periods of labeling, relatively more isotopic leucine, lysine, or valine is incorporated into the β-chain than into the α-chain when correction is made for the difference in the number of amino acid residues in each chain. If the labeling period is longer than 15 minutes, there is a near equality in the specific activity of the two chains, as might be expected (*3*). When reticulocytes from patients with acute leukemia in remission or with sickle cell anemia were incubated with radioactive amino acids, a similar phenomenon was observed. However, when reticulocytes obtained from patients with thalassemia major were used, a prominent difference was noted between the specific activities of the α- and β-chains after relatively long incubation periods. The α-chain consistently incorporated more carbon-14 per amino acid residue than the β-chain did.

Red cells were obtained by centrifugation from heparinized whole blood and washed once with cold Borsook's saline (*4*). The cells were then suspended in a modified Krebs-Henseleit medium (*2*) and incubated at 37°C for 2.5 minutes. The $C^{14}$-labeled amino acid was then added, and incubation was continued. The red cells were separated from the medium by centrifugation at 4°C, washed four times with ten volumes of Borsook's saline, then lysed in 0.0015*M* magnesium chloride solution. Particulate material was removed by centrifugation, first at 10,000*g* for 20 minutes, then at 100,000*g* for 60 minutes. The supernatant was dialyzed against 0.01*M* sodium phosphate, *p*H 7.0, containing potassium cyanide (100 mg/liter) for at least 24 hours, and the hemoglobin was purified on either columns of carboxylmethylcellulose (2 by 10 cm) or on columns of amberlite CG-50 (2 by 60 cm), the latter being used for the separation of hemoglobins A and F. After application of up to 500 mg of hemoglobin, the carboxylmethylcellulose column was washed with phosphate buffer, *p*H 7, and the hemoglobin was eluted with 0.05*M* dibasic sodium phosphate. Columns of amberlite were equilibrated with 0.05*M* sodium phosphate, 0.01*M* potassium cyanide, *p*H 7.02, before application of up to 600 mg of hemoglobin. After the columns had been washed free of readily elutable hemoglobin at 4°C, the buffer was changed to 0.075*M* sodium phosphate at room temperature. By this procedure hemoglobin A containing less than 5 percent of hemoglobin F was eluted.

Table 1. Clinical and experimental data on α- and β-chains of hemoglobin.

| Patient | Reticulocyte count (%) | Hgbn. purification* | Incubation (hr) | $C^{14}$ isotope | Radioactivity (count $min^{-1}$ $mg^{-1}$) α-chain | Radioactivity (count $min^{-1}$ $mg^{-1}$) β-chain | S.A.$_\alpha$/S.A.$_{\beta+}$ |
|---|---|---|---|---|---|---|---|
| | | | *Sickle cell disease* | | | | |
| M.G. | 8.2 | CM | 3½ | Valine | 226 | 359 | 0.63 |
| | | | *Acute leukemia (remission)* | | | | |
| J.H. | 17 | CM | 4 | Leucine | 234 | 297 | 0.79 |
| L.G. | 4.9 | CM | 3 | Valine | 62 | 68 | 0.91 |
| P.A. | 8.0 | CM | 2 | Leucine | 48 | 49 | 0.98 |
| | | | *Thalassemia major* | | | | |
| N.L. | 8.6 | CM | 3 | Valine | 183 | 98 | 1.9 |
| N.L. | 2.5 | Amb | 4 | Valine | 284 | 144 | 2.0 |
| J.L. | 3.4 | Amb | 4 | Valine | 464 | 201 | 2.3 |
| P.T. | 3.3 | Amb | 1 | Valine | 323 | 45 | 7.2 |

* CM, carboxymethylcellulose; Amb, amberlite. † S.A., specific activity.

Globin was prepared by adding the hemoglobin (10 to 30 mg/ml) by drops to a solution of 0.006*M* HCl in acetone at −20°C while stirring vigorously (*5*). The total volume of acetone was 10 to 20 times that of the hemoglobin (10 to 30 mg/ml) by drops to a solution of 0.006*M* HCl acid acetone, and dissolved in 11.7 percent formic acid. The α- and β-chains were then separated by the column chromatographic method of Chernoff (*6*). When samples contained both hemoglobins A and F, the γ-chain was eluted directly before the β-chain and could be separated adequately from the other peptide chains. The identity and purity of the peptide chains were verified by peptide mapping of tryptic digests (*7*). The separated α- and β-chains were dialyzed against distilled water to remove excess urea and then were lyophilized, and the protein content was determined by the Folin-Lowry method (*8*). Portions were plated on 2.5-cm stainless steel planchets, and radioactivity was assayed in a low-background gas flow beta-counter.

Patients J.L. and N.L. were siblings with thalassemia major; they had approximately 35 and 45 percent hemoglobin F, respectively, and 3.3 percent hemoglobin A2. Their mother's hemoglobin was 3 percent F and 4 percent A2, but the family was not otherwise studied. A thalassemic patient from a different family, P.T., had approximately 10 percent hemoglobin F.

In the patients with thalassemia (Table 1) the specific activity of the β-chain was approximately one-half that of the α-chain in the hemoglobin of two members of one family, regardless of the method of hemoglobin purification, and one-seventh that of the α-chain in one member of another family. This is in sharp contrast to patients in the remission phase of acute lymphocytic leukemia or patients who had sickle cell anemia, where in both instances the specific activity of the β-chain was always higher than or equal to that of the α-chain. Since the proportion of amino acid residues in the α-chain to those in β-chain are 12 : 18 for valine and 17 : 19 for leucine, the lower β-chain specific activity found in the patients with thalassemia is even more striking.

It remains to be determined whether this unusual labeling is specific for some forms of thalassemia as compared with other diseases with deficient hemoglobin synthesis. Conceivably, this pattern of isotopic labeling could be a manifestation of excess α-chain production by cells, a slow rate of β-chain assembly, or a loss of β-chain label. This latter could theoretically occur by dilution of labeled β-chain material with nonradioactive material prior to incorporation into hemoglobin.

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## Human Wart Virus: In vitro Cultivation

Abstract. *Inoculation of cell cultures of fetal skin of human and murine origin with virus extracted from human wart tissue resulted in the appearance of intracellular wart virus specific antigen, as demonstrated by fluorescent antibody techniques. Appearance of antigen was accompanied by cytopathogenicity and the accumulation of large numbers of characteristic virus particles.*

The viral etiology of human warts has been documented (*1*). The agent, 45 to 55 mμ in diameter, has been described as a DNA virus with 42 capsomeres in icosahedral symmetry (*2*). Because of its similarity to the rabbit papilloma, polyoma, and SV-40 viruses, the wart virus has been placed in the papova group (*3*).

The advantages of using a model human tumor (benign) virus system have attracted considerable attention to the human wart virus (*4*). However, there has been no unequivocal demonstration of cultivation of this agent in vivo or in vitro, other than in man (*5*). We now have presumptive evidence of the cultivation of the human wart virus in cell cultures of embryonic human and mouse skin.

Human warts (*Verruca vulgaris*) were removed surgically, immediately frozen in liquid nitrogen, and stored in sealed ampules at −70°C. Warts (at least six in each group) were pooled and pulverized in the frozen state. The resulting fine powder was suspended (100 mg/ml) in Hanks balanced salt solution (BSS) and homogenized in a glass homogenizer at 0°C. After centrifugation at 5000*g* for 10 minutes at 4°C the supernatant was put aside and the pellet was suspended to the original volume in BSS. The suspension was treated with high-frequency sound in a Raytheon oscillator (10 kcy/sec) at full output for 8 minutes. It was then centrifuged, and the supernatants of this and the previous centrifugation were pooled. Portions of these preparations were routinely examined for wart virus by electron microscopy; negatively stained (*6*) with phosphotungstic acid (PTA) they showed abundant characteristic human wart virus particles.

Antiserums were prepared in the rabbit against wart virus partially purified by differential centrifugation. The globulin fraction of pooled serums was obtained by precipitation with ammonium sulfate.

Almeida, Cinader, and Howatson (*7*) suggested that the specificity of immune globulins could be demonstrated by the formation of virus-antibody complexes as seen by electron microscopy. Accordingly, a portion of the immune globulin was mixed with an equal volume of the crude wart virus extract which was prepared from a wart pool separate from that used to immunize rabbits. The mixture was incubated at 37°C for 2 hours and then at 4°C overnight. After centrifugation at 1500*g*, the pellet was rinsed twice and suspended in phosphate-buffered saline (PBS). The preparation was stained with PTA and examined in the electron microscope. The complex resulting from the mixture of virus and immune globulin (Fig. 1) suggested the presence of wart virus specific antibody; this complex was not formed with normal rabbit serum globulin. The immune globulin was conjugated with fluorescein isothiocyanate (*8*), and the unconjugated dye was removed by gel-filtration on Sephadex G-25 (*9*).

Skin aseptically removed from a 4-month human fetus was washed, and a cell suspension was prepared by treatment with trypsin. Primary cultures were incubated for 6 days in Eagle's medium with 10 percent calf serum. Secondary cultures were prepared on coverslips in