References and Notes

1. J. Malý, *J. Inorg. Nucl. Chem. Letters*, in press. As early as 1949, G. T. Seaborg [in *The Transuranium Elements*, G. T. Seaborg, J. J. Katz, W. M. Manning, Eds. (McGraw-Hill, New York, 1949), p. 1516] predicted possible 2^+ oxidation states of elements 101 and 102.
2. J. J. Katz and G. T. Seaborg, *The Chemistry of the Actinide Elements* (Methuen, London, 1957), p. 415.
3. N. Edelstein, W. Easley, R. McLaughlin, *J. Chem. Phys.* **44**, 3130 (1966).
4. These half-lives are the best values from our measurements.
5. R. C. Vickery, *Chemistry of the Lanthanons* (Academic Press, New York, 1953). P. C. Stevenson and W. E. Nervik, *The Radiochemistry of the Rare Earths, Scandium, Yttrium, and Actinium*, Nuclear Science Series NAS-NS3020 (National Academy of Sciences–National Research Council, Washington, D.C., 1961), p. 12.
6. D. F. Peppard, E. P. Horwitz, G. W. Mason, in *Rare Earth Research*, J. F. Nachman and C. E. Lundin, Eds. (Gordon and Breach, New York, 1962), p. 15.
7. W. M. Latimer, *Oxidation Potentials* (Prentice-Hall, New York, ed. 2, 1952).
8. G. R. Choppin, B. G. Harvey, S. G. Thompson, *J. Inorg. Nucl. Chem.* **2**, 66 (1956).
9. E. S. Gureev, V. N. Kosyakov, G. N. Yakovlev, *Soviet Radiochemistry* **6**, 639 (1964).

10. We thank A. Ghiorso for the bombardments, R. W. Hoff for discussions, and B. J. Qualheim and J. E. Evans for assistance. This work was performed under the auspices of the U.S. Atomic Energy Commission.

14 August 1967

Rabbit Hemoglobin Biosynthesis: Use of Human Hemoglobin Chains To Study Molecule Completion

Abstract. *A cell-free protein-synthesizing system made from rabbit reticulocytes was used to incorporate ^{14}C-amino acids into hemoglobin. Electrophoretic analyses of the soluble products of this cell-free system revealed a fraction containing rabbit ^{14}C-alpha chains in addition to the rabbit ^{14}C-hemoglobin. The addition of isolated human hemoglobin beta chains to this system during active synthesis inhibited the release of newly synthesized rabbit ^{14}C-beta chains into solution from the ribosome fraction. This inhibition was possibly a result of hybrid hemoglobin formation between rabbit alpha and human beta chains. A model of hemoglobin construction in which soluble alpha chains are intermediates is suggested. These alpha chains may aid in the release of beta chains from the polyribosomes during the completion of the hemoglobin molecule.*

The hemoglobin molecule is composed of two peptide α-chains, two peptide β-chains, and four heme groups. Biosynthesis of the individual chains in rabbit reticulocytes, apparently occurring on separate polyribosomes, is followed by release to the soluble phase (*1*). However, the exact mechanism of construction of the final hemoglobin molecule remains unknown.

A cell-free system from rabbit reticulocytes can incorporate ^{14}C-amino acids into soluble hemoglobin (*2*). This protein-synthesis system should be useful in exploring the terminal steps in completion of hemoglobin. We wanted to obtain information on the nature of the soluble chains involved in completion.

Bucci and Fronticelli showed that isolated undenatured α- and β-chains can be obtained from human hemoglobin by treatment with *p*-chloromercuribenzoate (*3*). Because of the excellent homology between rabbit and adult human hemoglobin chains (*6*), we decided to see if the addition of these isolated human chains to the rabbit cell-free system (*4, 5*) would affect the completion of rabbit hemoglobin in a specific manner.

The presence of human hemoglobin α- or β-chains in the rabbit system throughout the 60-minute incubation did not change the total incorporation of ^{14}C-valine into protein (Table 1). However, human β-chains, but not human α-chains, caused a shift in distribution of radioactivity between the soluble and ribosome-bound proteins. The presence of human β-chains resulted in a 30-percent decrease in the amount of protein radioactivity in solution. There was a corresponding increase in the amount of protein radioactivity in the ribosome fraction. This effect was not seen if the β-chains were incubated with an equal amount of α-chains, thereby forming human hemoglobin A (*7*) before addition to the rabbit system. Thus, the change in distribution of radioactivity between soluble and ribosome-bound protein was caused specifically by free human β-chains.

Table 1. Effect of human hemoglobin chains on protein synthesis in a cell-free system from rabbit reticulocytes. In the reaction mixture, each assay contained 4 mg of ribosomes and 0.1 μmole of ^{14}C-valine (1.0 mc/mmole) in a final volume of 1.4 ml; 0.37 mg of human α-chains or 0.39 mg of human β-chains were included as indicated. The mixture was incubated for 60 minutes at 37°C. After the incubation, the ribosomes were removed by centrifugation (105,000*g* for 90 minutes). Both the ribosome pellet and resulting supernatant (soluble fraction) were washed for counting of radioactive protein in a liquid-scintillation system (*14*).

Addition to reaction mixture	^{14}C-valine in protein (mμmole)		
	Total	Soluble	Ribosomes
None	6.12	4.89	1.23
α-Chains	6.20	4.97	1.23
β-Chains	6.00	3.24	2.76

To learn whether this shift in distribution of protein radioactivity could be explained by an inhibition of nascent rabbit α- or β-chain release into solution from the ribosomes, we prepared rabbit globin from each of the two labeled soluble phases (supernatant after centrifugation at 105,000*g*) isolated after cell-free incubations with and without added human β-chains. The α- and β-chains of these globins were separated on carboxymethylcellulose columns (*8*). The presence of human β-chains in the incubation mixture caused a 45-percent decrease in the amount of radioactivity found in the rabbit β-chain peak. The amount of radioactivity in the rabbit α-chain remained the same. Thus, human β-chains appeared to inhibit selectively the release of newly synthesized rabbit β-, but not α-, chains into the soluble phase from the ribosome fraction (*9*).

We investigated the question of whether the human chains added during active synthesis were able to form hybrid hemoglobins (*10*) with the newly synthesized rabbit ^{14}C-chains. Forty percent of the soluble ^{14}C-protein radioactivity traveled with the carrier α_2^{RAB} β_2^{A} hybrid band when human β-chains had been present during active synthesis (Fig. 1a). Conversely, 55 percent of the soluble ^{14}C-protein radioactivity was located in the carrier α_2^{A} β_2^{RAB} band when human α-chains were present (Fig. 1b). Furthermore, chain-separation chromatography of the globins eluted from these hybrid bands showed that in both cases the ^{14}C radioactivity was located in the particular rabbit chain consistent with the formation of a true hybrid hemoglobin.

Formation of these hybrids was accompanied by a corresponding decrease in the amount of ^{14}C-protein radioactivity in the pure rabbit hemoglobin band. (See Fig. 2a for a typical electrophoretic pattern of the soluble phase from an incubation without added human chains.) Figure 1 also shows that the human chains did not induce the breakdown of already formed rabbit ^{3}H-hemoglobin included in the incuba-

tion mixtures. Collectively, these data suggest that human chains inhibit rabbit hemoglobin completion by forming complexes with the newly synthesized rabbit chains to form hybrid hemoglobins.

Formation of hybrid hemoglobin during active protein synthesis suggested the use of human chains for assay of rabbit ^{14}C-chains in solution after incubation of the cell-free system. The electrophoretic pattern (Fig. 2a) of the labeled soluble phase isolated after a 60-minute incubation in the absence of human chains shows a band of rabbit ^{14}C-hemoglobin containing 67 percent of the radioactivity of the soluble protein. Moreover, Fig. 2a indicates the presence of some electrophoretically disperse, labeled material traveling primarily between the line of application and the rabbit hemoglobin band.

A sample of this isolated soluble phase was incubated with human β-chains. Subsequent electrophoresis revealed that most of the disperse ^{14}C-protein had been converted into a material traveling with the α_2^{RAB} β_2^{A} hybrid hemoglobin (Fig. 2b). Figure 2 also shows that neither the newly synthesized rabbit ^{14}C-hemoglobin nor already formed rabbit ^{3}H-hemoglobin was broken down during this assay with human β-chains. Chain-separation chromatography of the globin eluted from the hybrid band of Fig. 2b showed that only the α-chain was ^{14}C-labeled. We conclude from these data that soluble, newly synthesized α-chains exist in a nonhemoglobin form after protein synthesis has occurred in the cell-free system.

The reason for the electrophoretically disperse nature of this α-chain component is unknown. Perhaps the lack of enough protein to act as carrier prevents the α-chains from forming a discrete band. The newly released α-chains might also exist in association with other soluble components. Other studies with whole reticulocyte cells in this laboratory have revealed a similar soluble component containing newly synthesized α-chains which appear to be intermediates in hemoglobin construction (*11*).

When another sample of the soluble phase of the experiment (Fig. 2) was incubated with human α-chains instead, no ^{14}C-α_2^{A} β_2^{RAB} hybrid was detected in the electrophoretic pattern. This suggests that the soluble phase isolated after synthesis did not contain measur-

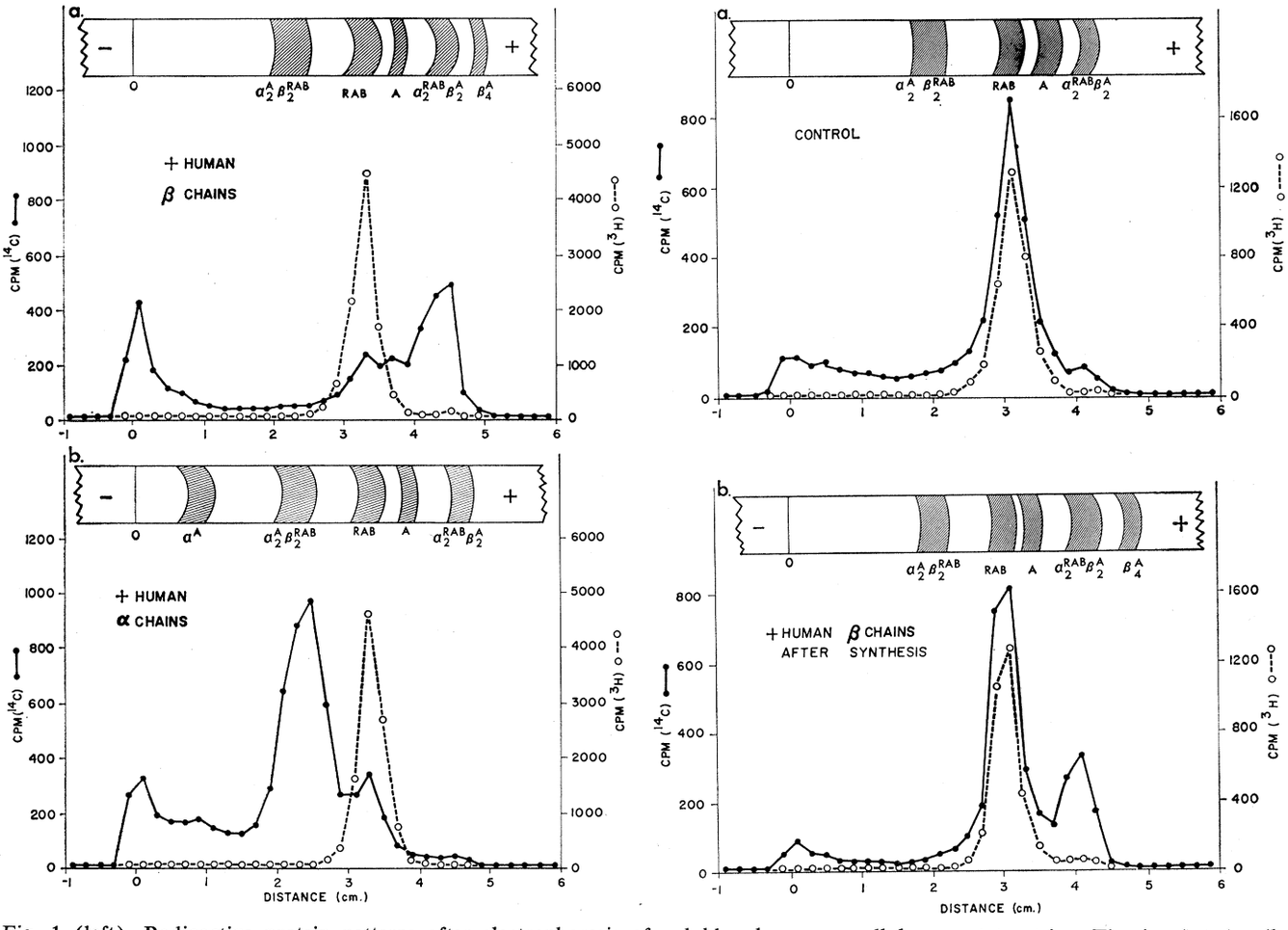

Fig. 1 (left). Radioactive protein patterns after electrophoresis of soluble phases on cellulose acetate strips. The incubated cell-free mixtures (*4*) contained (a) 0.37 mg of human hemoglobin β-chains and (b) 0.38 mg of human hemoglobin α-chains. The assays also included 2.3 mg of rabbit ^{3}H-hemoglobin prepared from reticulocyte cells labeled with ^{3}H-leucine. After incubation for 60 minutes, 15 mg (0.1 ml) of a hybrid (rabbit and human) hemoglobin mixture (*10*) was added as carrier protein. The ribosomes were removed by centrifugation, and the supernatant was analyzed by electrophoresis (*15*). The position of the stained bands of the carrier hybrid hemoglobin mixture are shown with each radioactive protein pattern. The line of application is denoted by zero. Fig. 2 (right). Electrophoretic patterns of (a) the soluble phase isolated after a cell-free incubation and (b) the same soluble phase incubated with human hemoglobin β-chains. Fractions (0.15 ml) of the soluble phase were mixed with 0.05 ml containing (a) phosphate buffer and (b) 0.19 mg of human β-chains. These assays were incubated for 10 minutes at 37°C and analyzed by electrophoresis (*15*). For other details, see the legend to Fig. 1.

able amounts of free rabbit ^{14}C-β-chains. Additional studies showed that 90 percent of the ^{14}C radioactivity in the pure rabbit hemoglobin band of Fig. 2 was located in the β-chain. This observation implies that during protein synthesis unlabeled rabbit α-chains combine with newly synthesized ^{14}C-β-chains to form completed rabbit hemoglobin. Presumably, these rabbit α-chains are endogenous to the ribosome and enzyme fractions of the incubation mixture.

We suggest that, in rabbit hemoglobin synthesis, growing α-chains are released from the polyribosomes to the solution when their primary-sequence synthesis is completed. Synthesized β-chains are brought into the molecule finally by complementation with these α-chains. Complementation may occur with the β-chains still growing on the polysome. Colombo and Baglioni have shown that polysomes isolated from reticulocytes contain about one complete α-chain for every 5.5 being synthesized (*12*). Others have suggested that soluble α-chains might form complexes with growing β-chains on the polysome and aid in their completion and release (*13*). Therefore, in our experiments addition of human β-chains to the cell-free system during active protein synthesis might indirectly inhibit release of rabbit β-chains by depleting the supply of rabbit α-chains through α_2^{RAB} β_2^{A} hybrid formation. However, there also exist alternative explanations for the observed effect of human β-chains on the release of rabbit β-chains.

JOSEPH R. SHAEFFER
PATRICIA K. TROSTLE
RICHARD F. EVANS*
Department of Physics, M. D. Anderson Hospital and Tumor Institute, and
University of Texas Graduate School of Biomedical Sciences, Houston 77025

References and Notes

1. J. R. Warner, A. Rich, C. E. Hall, *Science* **138**, 1399 (1962).
2. R. Schweet, H. Lamfrom, E. Allen, *Proc. Nat. Acad. Sci. U.S.* **44**, 1029 (1958).
3. E. Bucci and C. Fronticelli, *J. Biol. Chem.* **240**, PC551 (1965).
4. The rabbit reticulocyte cell-free system was reconstituted according to E. H. Allen and R. S. Schweet [*J. Biol. Chem.* **237**, 760 (1962)]. The usual mixture contained 2.4 mg of ribosomes, receiving a single centrifugation and 3 mg of a soluble enzyme fraction purified by ammonium sulfate precipitation at *p*H 6.5 (*5*) in a final volume of 0.84 ml. Each assay contained 0.025 μmole of L-^{14}C-leucine (231 mc/mmole) and 0.025 μmole of L-^{14}C-valine (208.5 mc/mmole); incubation at 37°C for 60 minutes.
5. R. Arlinghaus, J. Shaeffer, R. Schweet, *Proc. Nat. Acad. Sci. U.S.* **51**, 1291 (1964).
6. G. Braunitzer, J. S. Best, U. Flamm, B. Schrank, *Z. Physiol. Chem.* **346**, 207 (1966); G. von Ehrenstein, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 705 (1966).
7. E. Bucci, C. Fronticelli, E. Chiancone, J. Wyman, E. Antonini, A. Rossi-Fanelli, *J. Mol. Biol.* **12**, 183 (1965).
8. H. M. Dintzis, *Proc. Nat. Acad. Sci. U.S.* **47**, 247 (1961).
9. Chain separation of the globin from the incubation without added human chains revealed that 29 percent of the radioactivity was in the α-chain peak, and 71 percent was in the β-chain peak. The reason for this disparity in the radioactivity of the soluble chains of the cell free system is not yet clear.
10. H. A. Itano and S. J. Singer, *Proc. Nat. Acad. Sci. U.S.* **44**, 522 (1958); D. Labie, J. Rosa, J. C. Dreyfus, G. Schapira, *Nature* **194**, 384 (1962).
11. J. R. Shaeffer, *Biochem. Biophys. Res. Commun.* **28**, 647 (1967).
12. B. Colombo and C. Baglioni, *J. Mol. Biol.* **16**, 51 (1966).
13. A. L. Cline and R. M. Bock, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 321 (1966); A. L. Shapiro, M. D. Scharff, J. V. Maizel, Jr., J. W. Uhr, *Proc. Nat. Acad. Sci. U.S.* **56**, 216 (1966).
14. J. Shaeffer, G. Favelukes, R. Schweet, *Biochim. Biophys. Acta* **80**, 247 (1964).
15. For electrophoresis, 5 μl of the sample was applied to a cellulose acetate strip (2.5 × 17.1 cm) (Sepraphore III, Gelman Instrument Co.) wet with the electrophoresis buffer (tris-barbital-sodium barbital, *p*H 8.8; ionic strength = 0.025). The electrophoresis was run at 300 volts (approximately 1.5 ma per strip) and 4°C for 90 minutes. The strips were stained with Ponceau S in 5-percent trichloroacetic acid, washed with 5-percent acetic acid, and allowed to dry. The strips were cut up in sections (0.20 cm wide) for liquid-scintillation counting (*14*). In certain experiments, globin was eluted from unstained strips.
16. Supported by PHS institutional grant FR 05511 IN-44 and NSF grant GB 5890.
* Present address: Department of Biochemistry, College of Biological Sciences, University of Minnesota, St. Paul 55101.
31 August 1967

"Blastokinin": Inducer and Regulator of Blastocyst Development in the Rabbit Uterus

Abstract. *A protein fraction that induces blastulation of rabbit morula and stimulates blastocyst development has been isolated from the uterus of the rabbit at early pregnancy. Partial purification of this fraction indicates that the activity is restricted to a single homogeneous protein component. The term "blastokinin" is proposed to describe the compound.*

Development of mammalian ova in vitro is arrested by the early blastocyst stage (*1, 2*). Even under the most favorable culture conditions known, only initially cavitated blastocysts have been obtained from fertilized rabbit ova or any intermediate cleavage stage in vitro. Rarely any progress much beyond this stage has been noticed. Typically, 3-day rabbit morulae will grow to the stage of initial cavitation in 12 to 24 hours in media supplemented with serum; thereafter they cease to grow, or they expand very slowly over a period of several days. However, somewhat older embryos (5- to 6-day blastocysts) can be grown in vitro for several days but at a growth rate that continues to decline relative to that occurring in vivo (*3, 4*). Although slightly better growth rates have been obtained with each new modification of the medium, it has not yet been possible to get continued growth of blastocysts, nor growth of cleavage-stage embryos beyond initial cavitation, comparable to the normal.

Obviously, the uterine environment in some way provides for the regulation of these phenomena. This regulation may be brought about by a single component or may be the result of proper balancing in the uterus of other, more common components of relatively small molecular size, inadequately provided for in the culture media. So far as is known, all evidence tends to exclude the latter possibility. The inference then follows that, should there be such a regulator, it is probably a macromolecular component. We present evidence that such a component occurring at the time of blastulation and blastocyst development can induce and regulate these processes.

Table 1. Effect of supplementation of Ham's F10 with "blastokinin" on growth and development of 3-day rabbit morulae into blastocysts, compared to that of supplementation with other proteins.

Protein concentration (mg/ml)	Embryos used (No.)	Cavitated in 24 hr (No.)	Expanded in 24 hr (No.)
Control (Ham's F10)			
	24	12	(1?)
"Blastokinin"			
0.05	10	10	0
.10	10	10 (2?)	2 (1?)
.20	14	13	10
.40	10	10	6
0.60	11	6	4 (1?)
1.00	10	5	3
2.00	6	0	0
10.00	6	0	0
Complete uterine-fluid protein, 5 days after coitus			
0.30	10	5	0
.50	14	12	11
1.00	12	4	2
3.00	8	5	0
10.00	11	8	0
Maternal serum proteins			
0.30	10	8	0
3.00	12	10	(2?)