acid; CTP or cyt-P-P-P, ATP, UTP, and GTP for the tri- and CDP, ADP, UDP, and GDP for the di- and CMP, AMP, UMP, and GMP for the monophosphates of cytidine, adenosine, uridine, and guanosine; Tris, tris-(hydroxymethyl)-aminomethane; TCA, trichloroacetic acid; c.p.m., counts per minute; Pi, inorganic orthophosphate; P-O-P, inorganic pyrophosphate.

[5] Kornberg, A., I. R. Lehman, and E. S. Simms, *Federation Proc.*, **15**, 291 (1956).

[6] Kornberg, A., in *The Chemical Basis of Heredity*, ed. W. D. McElroy and B. Glass (Baltimore: Johns Hopkins Press, 1957), p. 579.

[7] Bollum, F. J., and V. R. Potter, *Abstr. Am. Chem. Soc.*, 132nd meeting, 19C (1957).

[8] Heidelberger, C., E. Harbers, K. C. Leibman, Y. Takagi, and V. R. Potter, *Biochim. et Biophys. Acta*, **20**, 445 (1956).

[9] Paterson, A. R. P., and G. A. LePage, *Cancer Research*, **17**, 409 (1957).

[10] Canellakis, F. S., *Biochim. et Biophys. Acta*, **25**, 217 (1957).

[11] Edmonds, M., and R. Abrams, *Biochim. et Biophys. Acta*, **26**, 226 (1957).

[12] Herbert, E., *J. Biol. Chem.*, **231**, 975 (1958).

[13] Hecht, L. I., P. C. Zamecnik, M. L. Stephenson, and J. F. Scott, *J. Biol. Chem.*, **233**, 954 (1958).

[14] Hoagland, M. B., P. C. Zamecnik, and M. L. Stephenson, *Biochim. et Biophys. Acta*, **24**, 215 (1957).

[15] Berg, P., and E. J. Ofengand, these PROCEEDINGS, **44**, 78 (1958).

[16] Weiss, S. B., and L. Gladstone, *J. Am. Chem. Soc.*, **81**, 4118 (1959).

[17] Baddiley, J., J. G. Buchanan, and A. R. Sanderson, *J. Chem. Soc.*, 3107 (1958).

[18] Kennedy, E. P., and S. B. Weiss, *J. Biol. Chem.*, **222**, 193 (1956).

[19] Lieberman, I., A. Kornberg, and E. S. Simms, *J. Biol. Chem.*, **215**, 429 (1955).

[20] Lehman, I. R., M. J. Bessman, E. S. Simms, and A. Kornberg, *J. Biol. Chem.*, **233**, 163 (1958).

[21] Lohman, K., *Biochem. Z.*, **254**, 381 (1932).

[22] Bessman, M. J., I. R. Lehman, J. Adler, S. B. Zimmerman, E. S. Simms, and A. Kornberg, these PROCEEDINGS, **44**, 633 (1958).

[23] Goldwasser, E., personal communication.

[24] Kirby, K. S., *Biochem. J.*, **64**, 405 (1956).

[25] Gierer, A., and G. Schramm, *Nature*, **177**, 702 (1956).

[26] Gomori, G., *J. Lab. Clin. Med.*, **27**, 955 (1942).

[27] Borkenhagen, L. F., and E. P. Kennedy, *J. Biol. Chem.*, **234**, 849 (1959).

[28] Lowry, W., *J. Biol. Chem.*, **193**, 265 (1951).

[29] Razzell, W. E., and H. G. Khorana, *J. Biol. Chem.*, **234**, 2114 (1959).

[30] Preiss, J., and P. Berg, *Federation Proc.*, **19**, 317 (1960).

[31] Goldwasser, E., *J. Am. Chem. Soc.*, **77**, 6083 (1955).

[32] Edmonds, M., and R. Abrams, *Federation Proc.*, **19**, 317 (1960).

FORMATION OF THE PEPTIDE CHAIN OF HEMOGLOBIN

BY JOHN BISHOP,* JOHN LEAHY, AND RICHARD SCHWEET*†

BIOCHEMISTRY DEPARTMENT, CITY OF HOPE MEDICAL CENTER, DUARTE, CALIFORNIA

Communicated by James F. Bonner, June 30, 1960

The cytoplasmic ribonucleoprotein particles, or ribosomes, are now considered to be the major site of protein synthesis in a variety of tissues.[1] Kinetic studies with intact reticulocytes have indicated that these particles are the site of hemoglobin synthesis.[2, 3] Little is known, however, about the intra-ribosomal events which lead eventually to the final peptide chain. Two general mechanisms may be distinguished: simultaneous linkage of amino acids, and stepwise synthesis involving intermediate peptides. Intermediate peptides might be formed sequentially

from one end of the chain, or clusters of amino acids might form peptides randomly along the chain and then join to complete it. The evidence supporting these two hypotheses has been discussed by Steinberg *et al.*[4] and by Loftfield.[5]

More direct information on this problem in the case of hemoglobin synthesis has been obtained using a cell-free system.[6] The present approach was suggested by the finding that when ribosomes containing C^{14}-labeled amino acids were incubated with unlabeled amino acids together with the necessary enzymes and cofactors, the ribosome-bound C^{14}-amino acids were transferred to and found in hemoglobin.[7] These experiments indicated that hemoglobin precursors, which appear to be peptides, are present in ribosomes, and are released as completed hemoglobin chains.

In the experiments reported here, ribosomes labeled with C^{14}-valine (the N-terminal amino acid of hemoglobin) were isolated from intact cells, and then incubated with C^{12}-amino acids in the cell-free system. The labeled hemoglobin formed was then isolated. In other experiments, unlabeled ribosomes were incubated with C^{14}-valine and the labeled hemoglobin isolated. The radioactivity in N-terminal valine and in valine in the rest of the hemoglobin chain was then compared. The results strongly suggest that the hemoglobin chain is synthesized sequentially starting at the N-terminal end, and that a pool of incomplete hemoglobin chains, containing N-terminal valine and variable amounts of the rest of the chain, is present in ribosomes.

Methods and Materials.—Enzymes used were prepared from the soluble fraction of reticulocyte lysates by ammonium sulfate fractionation.[8] The uniformly-labeled C^{14}-amino acids used contained 2.4×10^6 counts per min per μmole.

Intact reticulocyte experiments: Washed rabbit reticulocytes were prepared and incubated essentially as described by Borsook *et al.*[9] Each ml of packed reticulocytes was incubated with 0.6 ml of a mixture of C^{12}-amino acids,[10] 0.2 ml of fresh plasma, 0.04 ml of 1 *M* Tris,[11] pH 7.5, 0.04 ml of 0.01 *M* ferrous ammonium sulfate, 1.1 ml of twice-concentrated, modified Krebs-Henseleit solution,[10] 0.2 ml of 0.001 *M* C^{14}-valine or C^{14}-leucine, and water to a final volume of 4 ml. The mixture was incubated at 37° for 10 min, chilled, and the cells washed. Ribosomes were then prepared in the usual manner,[6, 8] except that 20 μmoles of C^{12}-valine was added to the wash and also again after lysis of the cells. The soluble protein fraction was used to prepare hemoglobin.

Cell-free experiments: Ribosomes prepared from labeled or unlabeled cells were usually washed twice and incubated with enzymes, nucleotides, and other constituents as previously described.[6, 12] All incubation mixtures differed only in containing C^{14}- or C^{12}-amino acids as indicated. After incubation at 37° for 10 min, the mixtures were chilled and 50 to 100 mg of crude carrier hemoglobin was added. Ribosomes were separated by centrifugation at $105{,}000 \times g$ for 1 hr. The second cell-free incubation, when employed, was for 20 min, using once-washed ribosomes separated as described above. Ribosomes from this incubation were again separated by centrifugation. Aliquots of ribosomes and supernatant were counted after each incubation in the usual manner.[6]

Preparation of hemoglobin for N-terminal analysis: Hemoglobin was prepared for N-terminal analysis from incubation supernatants by removal of a pH 5 fraction followed by precipitation between 45 and 90 per cent saturation

with ammonium sulfate. Results were not altered when hemoglobin was purified further by chromatography on IRC-50.[6]

The 45 to 90 per cent ammonium sulfate precipitate was dialyzed against 0.1 *M* Tris, pH 7.5. The protein was then precipitated with 5 per cent TCA (at room temperature) and washed with 5 per cent TCA and then with 1 per cent TCA. The precipitate was dissolved in 0.1 *N* sodium hydroxide at a final concentration of 10 to 15 mg protein per ml and taken to pH 9.5 with saturated boric acid. This procedure yielded a washed protein in *solution* buffered at the pH used for N-terminal analysis.

Preparation of DNP-derivatives: The protein solution (containing 1,000 to 20,000 cpm) was mixed with 2 volumes of 2.5 per cent (v/v) dinitrofluorobenzene in 95 per cent ethanol[13] and shaken for 2 hr at 40°. The insoluble DNP-protein was centrifuged off, washed successively with 0.5 *N* hydrochloric acid, ethanol-ether (1–2 v/v), and ether, and dried at 40°. The dry DNP-protein was hydrolyzed for 24 hr at 110° in constant-boiling hydrochloric acid (5 to 10 mg protein per ml). The DNP amino acids were separated quantitatively from the water-soluble amino acids. Extreme care was taken to remove traces of free amino acids from the DNP-valine. Paper chromatography using 2 solvents[14, 15] showed that the radioactivity was present as DNP-valine.

Preparation of PTH-derivatives: A similar aliquot of the protein solution was mixed with an equal volume of dioxane and one-twentieth volume of phenylisothiocyanate[16] and shaken for 2 hr at 40°. The protein was precipitated with 5 per cent TCA and centrifuged off. The precipitate was washed with ethanol-ether (1–2 v/v), benzene, and ether, and dried. The PTC-protein was suspended in 1*N* hydrochloric acid (5 to 10 mg protein per ml) and hydrolyzed for 1 hr at 110°. The hydrolysate was treated in the same way as the DNP-protein hydrolysate.

Aliquots of water-soluble and ether-soluble fractions from both determinations were pipetted onto planchets containing Whatman No. 1 filter paper, dried and counted in a gas-flow counter with Micromil window.

Experiments and Results.—To test the analytical methods, hemoglobin labeled in the intact cell with C^{14}-valine, was studied (Table 1). In 8 separate experiments the radioactivity found in N-terminal valine was 8.4 ± 1.1 (standard deviation) per cent of the total radioactivity in the isolated hemoglobin by the DNP method

TABLE 1

N-TERMINAL RADIOACTIVITY IN WHOLE-CELL HEMOGLOBIN*

	DNP method			PTH method		
C^{14}-amino acid used	Ether layer cpm	Water layer cpm	Per cent N-terminal C^{14}-amino acid	Ether layer cpm	Water layer cpm	Per cent N-terminal C^{14}-amino acid†
Valine	665	7,611	8.3	725	7,660	8.6
Leucine	40	20,090	0.2	60	17,955	0.33

* Intact cells were incubated for 15 min with the indicated C^{14}-amino acid as described in the text. The hemoglobin was isolated and radioactivity in the N-terminal amino acid determined.

† The per cent is the radioactivity in the ether layer divided by the total radioactivity. The results are given for a typical experiment.

and 8.2 ± 0.6 per cent by the PTH method. These values correspond closely to the calculated value based on the composition of rabbit hemoglobin, which contains approximately 46 valine residues per molecule,[17] of which 4 valine residues.[18]

or 8.7 per cent, are N-terminal. The per cent N-terminal C^{14}-valine found, therefore, is the amount expected from uniformly-labeled hemoglobin, and is in agreement with the original report of Kruh and Borsook[19] that reticulocytes synthesize hemoglobin *in vitro*. When C^{14}-leucine was used, a negligible amount of radioactivity was found as N-terminal amino acid, as expected (Table 1).

When hemoglobin was isolated after the usual cell-free incubation of unlabeled ribosomes with C^{14}-valine, however, no radioactivity was found in N-terminal valine. This surprising result, which was confirmed in a number of experiments, suggested the possibility shown in Figure 1.

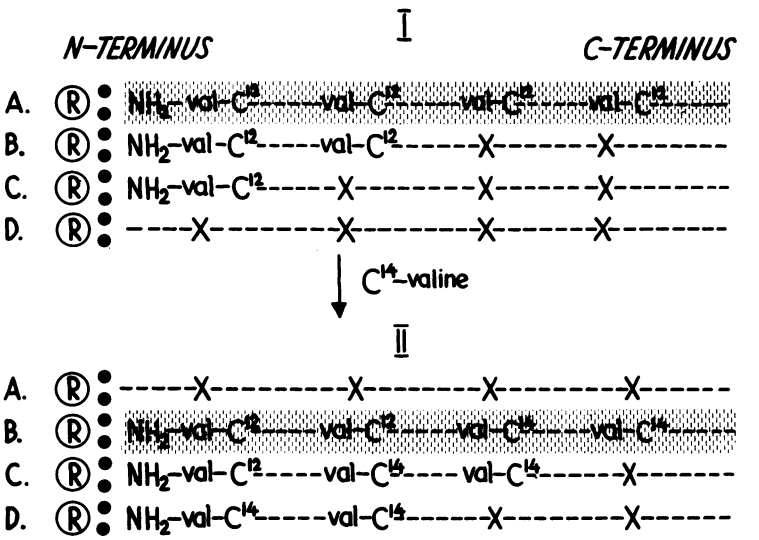

FIG. 1.—Proposed peptides in ribosomes and the growing peptide chain. *R*, ribosome; : —indicates the attachment of the N-terminal valine; *X*, unoccupied valine site. *I*, ribosomes as isolated; *II*, the same ribosomes after brief incubation with C^{14}-valine. The shaded area indicates a completed chain.

In this figure, IA to ID represent various ribosomes which are present in any cell. In IA, the ribosome contains a completed hemoglobin chain, ready to be released. In IB and IC, chains in various stages of completion are present. These represent the condition of most of the ribosomes. In ID, the completed chain has just been released and the template is unoccupied.

The stages in the second part of the figure show the same ribosomes after a few C^{14}-valine residues have been added. In IIA, the completed chain has been released, but this hemoglobin is unlabeled. When this ribosome forms and releases another molecule of hemoglobin (not shown) this hemoglobin will be completely labeled and will contain 8.4 per cent of the radioactivity in N-terminal valine. In IIB and C, C^{14}-valine has been added to the chains and when these are completed and released, they will be labeled, but contain *no radioactivity* in N-terminal valine. In IID, a chain containing N-terminal C^{14}-valine is being formed. However, these originally empty ribosomes will be the last to release completed hemoglobin and little hemoglobin of this type will be formed at first.

If we assume that in the *cell-free system* most of the ribosomes are able to com-

plete the peptide chain which is present, but not to initiate the synthesis of a second chain, that is, "turn-over" only once, labeled hemoglobin with little or no radioactivity in N-terminal valine will be formed in short incubations. The suggestion that the lesion in the cell-free system, which limits the amount of hemoglobin synthesis, is the inability of the ribosomes to "turn-over" is supported by several lines of evidence. For example, the specific activity of *ribosomes* isolated after incubation in the cell-free system is about 50 per cent of ribosomes isolated after labeling in the intact reticulocyte.[3] However, hemoglobin isolated from the intact cell has 500 times the radioactivity of the hemoglobin produced by an equivalent amount of ribosomes incubated in the cell-free system. It appears that in the cell-free system ribosomes *start synthesis* in a similar way to the *intact cell*, but either complete and release hemoglobin chains very slowly, or cannot start a completely new chain readily.

The hypothesis shown in Figure 1 was tested with labeled ribosomes. Intact cells were incubated with C^{14}-valine under conditions such that each ribosome had "turned-over" many times. Ribosomes isolated from such cells should contain only *completely labeled* peptides. That is, the ribosomes would contain peptides as shown in the figure (part I), but C^{14}-valine would be present in all cases. When these ribosomes are incubated with C^{12}-valine, the completed hemoglobin chains should contain *more* than 8.5 per cent of their radioactivity in N-terminal valine, since the chains are completed with C^{12}-valine. The results of 4 separate experiments (Table 2) gave an average of 12.8 per cent radioactivity in N-terminal valine. These results provide strong support for the proposed mechanism. The possibility that these labeling patterns were the result of the inability of the cell-free system to produce a completed hemoglobin chain was largely excluded by other experiments, in which ribosomes labeled with *C^{14}-valine* in the intact cell were incubated with *C^{14}-valine* in the cell-free system (Table 2). This should correspond to complete labeling of the molecule, as in whole cell hemoglobin. The value of 8.4 per cent N-terminal valine which was found is the expected value. When ribosomes labeled with C^{14}-leucine in the whole cell were used, no N-terminal radioactivity was found in the hemoglobin isolated after cell free incubation (Table 2).

TABLE 2

N-TERMINAL RADIOACTIVITY IN HEMOGLOBIN AFTER CELL-FREE INCUBATION

Condition of ribosomes	Cell-free incubation	Per cent N-terminal C^{14}-amino acid — DNP method	PTH method	Average
Unlabeled*	With C^{14}-valine	0	0	0
Labeled with C^{14}-valine†	With C^{12}-valine	11.9	13.2	12.6
Labeled with C^{14}-valine	With C^{12}-valine	14.1	10.0	12.0
Labeled with C^{14}-valine	With C^{12}-valine	14.2	12.3	13.3
Labeled with C^{14}-valine	With C^{12}-valine	12.9	13.1	13.0
Labeled with C^{14}-valine	With C^{14}-valine‡	8.7	8.1	8.4
Labeled with C^{14}-leucine	With C^{12}-leucine	0	0.5	0.25

* Ribosomes were isolated in the standard way and incubated for 10 min under standard assay conditions and the hemoglobin isolated and analyzed.

† *Intact reticulocytes* were incubated with C^{14}-valine or C^{14}-leucine as described in the text and then the ribosomes were isolated and incubated in the cell-free system in the standard way with the indicated amino acid. The hemoglobin was isolated and analyzed.

‡ Average of two experiments.

If peptides of various sizes were present in ribosomes, as illustrated in Figure 1, it appeared likely that larger peptides would be completed first. Thus, if ribosomes containing C^{14}-labeled peptides were incubated with C^{12}-amino acids, the

hemoglobin formed at first would be almost completely labeled. The hemoglobin formed later would come from ribosomes containing smaller labeled peptides, and would contain a higher percentage of N-terminal valine. The idea was tested by incubating ribosomes labeled with C^{14}-valine with C^{12}-amino acids as before. The ribosomes *re-isolated* after this incubation should contain shorter peptide chains. These ribosomes were then incubated a second time with C^{12}-amino acids. The hemoglobin isolated in this case contained 15.9 per cent N-terminal C^{14}-valine (Table 3), in contrast to the average of 12.8 per cent found after the first incubation.

TABLE 3

N-TERMINAL RADIOACTIVITY IN HEMOGLOBIN AFTER DOUBLE CELL-FREE INCUBATION

Condition of ribosomes	First cell-free incubation	Second cell-free incubation	Per Cent N-Terminal C^{14}-Valine		
			DNP method	PTH method	Average
Labeled with C^{14}-valine	With C^{12}-valine†	With C^{12}-valine	15.9	14.6	15.2
Labeled with C^{14}-valine	With C^{12}-valine	With C^{12}-valine	17.1	16.1	16.6
Unlabeled*	With C^{14}-valine	With C^{12}-valine	6.5	8.5	7.5

* These results are the average of two experiments. In three other experiments little or no N-terminal valine was found.

† Determination of the percentage of N-terminal valine in the hemoglobin from the first cell-free incubation of these ribosomes gave values similar to those in Table 2.

An estimate of the average length of the incomplete chain in the ribosome can be made from the data. For example, 12.8 per cent of N-terminal valine corresponds to one N-terminus for each 7 internal valines, while the result with whole-cell hemoglobin is one N-terminus for each 11 internal valines. Thus, the incomplete chain in these ribosomes is calculated to be 63 per cent of a complete hemoglobin chain. The hemoglobin isolated after the second incubation which contained 15.9 per cent N-terminal C^{14}-valine, was derived from an incomplete chain in the ribosome which was 45 per cent of the finished chain. The average length of the incomplete hemoglobin chain in ribosomes in the steady state appears to be approximately 50 per cent of the completed chain.[20] These results, however, provide no information as to the number of chains synthesized per ribosome.

The evidence that ribosomes containing shorter incomplete chains could be isolated after a cell-free incubation led to an attempt to demonstrate N-terminal C^{14}-valine in hemoglobin derived from ribosomes labeled originally in the *cell-free system.* It appeared likely that ribosomes labeled in the cell-free system might include a few which contained N-terminal valine. These would be ribosomes which were "empty" when isolated (ID, Fig. 1), or ribosomes which had "turned-over" more than once in the cell-free system. The hemoglobin of the first cell-free incubation might not contain any hemoglobin derived from such ribosomes. However, the ribosomes isolated from this incubation and re-incubated with C^{12}-amino acids might yield some hemoglobin from such ribosomes. In addition, the percentage of N-terminal C^{14}-valine would be accentuated. This was the case, and N-terminal valine was found (Table 3) in hemoglobin formed completely in the cell-free system. In three other experiments, no N-terminal valine was found. The experiments which gave N-terminal valine in the cell-free system, however, were those in which the ribosomes were most active, that is, released a larger amount of labeled hemoglobin per weight of ribosome.

These experiments indicate that under favorable conditions the complete pep-

tide chain of hemoglobin is synthesized in the cell-free system. Under most conditions it is likely that some of the hemoglobin chains labeled in the cell-free system contain N-terminal C^{14}-valine, but that these are difficult to detect in the presence of the large amount of C^{14}-valine which is not N-terminal. It is probable, also, that incubation times longer than those used in these experiments would be more likely to release hemoglobin containing N-terminal C^{14}-valine from the ribosomes.

Another prediction of the hypothesis that hemoglobin chains are synthesized starting from the amino terminal end, is that hemoglobin isolated from the *intact cell* after short incubations should contain a low percentage of N-terminal C^{14}-valine. If the ribosomes in the intact cell only "turn-over" a few times the hemoglobin formed should resemble hemoglobin of the cell-free system and have low N-terminal C^{14}-valine. Preliminary experiments with intact cells incubated with C^{14}-valine at 37° for short periods of time (0.5 to 2 min) have given hemoglobins with N-terminal C^{14}-valine ranging from 2.5 per cent to the equilibrium value of 8.3 per cent. Further, the per cent of N-terminal C^{14}-valine has been proportional to the number of "turn-overs" of the ribosomes. The number of "turn-overs" was considered to be the ratio of total radioactivity in the incubation supernatant to that in the ribosomes.

Discussion and Summary.—The concept, that ribosomes contain incomplete peptide chains of the protein they are producing, has been discussed by Loftfield *et al.*[21] and a figure similar to the one used here has been presented by these authors. Dintzis *et al.*[3] have suggested that ribosomes from rabbit reticulocytes contain incomplete hemoglobin chains. Early studies with the cell-free system[6] provided further evidence based on the amounts of various C^{14}-amino acids incorporated into ribosomes. These amounts were similar to those in hemoglobin. More recently, C^{14}-labeled ribosomes incubated in the presence of unlabeled amino acids were shown to transfer radioactivity to hemoglobin.[7] The amount of radioactivity transferred to soluble protein corresponded to the amount present in the ribosomes after acid and alkali washing and extraction with ethanol-ether. The properties of the intermediates are those of peptides bound firmly to the ribosome. The data presented here indicates that the ribosome-bound intermediates are incomplete chains starting with N-terminal valine.

The results which lead to the conclusion that the peptide chain is synthesized from the amino terminus and that incomplete sequences are present in the ribosome are: (1) The incubation of unlabeled ribosomes with C^{14}-valine in the cell-free system gave very low N-terminal C^{14}-valine. (2) The incubation of C^{14}-valine-labeled ribosomes with C^{12}-valine in the cell-free system gave an excess of N-terminal C^{14}-valine. An important control, which demonstrated that these results were not due to release of partial peptide chains or to other lesions in the system, was provided by the incubation of C^{14}-valine-labeled ribosomes with C^{14}-valine in the cell-free system. In this case, the percentage of N-terminal C^{14}-valine was that of hemoglobin synthesized in the intact cell. (3) A low percentage of N-terminal C^{14}-valine was found in hemoglobin made in the *intact* reticulocyte in the first few minutes of incubation.

We have attempted to explain these results by alternative hypotheses. One of these, discussed previously by Steinberg,[4] involves different sizes of intermediate

pools. However, in these studies, such pools would have to be bound to the ribosome, resistant to dilute acid and alkaline hydrolysis and ethanol-ether extraction. In addition, the pool which leads to the N-terminus would have to be quite different from the average of the other pools, and other assumptions would have to be invoked. Studies of the radioactivity in the carboxyl terminus of hemoglobin and also of the specific activity of valine residues in known locations along the chain should provide a further test of the mechanism proposed here. However, a definitive test may require isolation of the proposed peptides from the ribosomes. Dintzis *et al*[3] have reported preliminary studies on isolation of peptides from reticulocyte ribosomes.

If this mechanism is correct, it seems likely that the N-terminal amino acid is held by some rather firm linkage to the ribosome, probably through its amino group. The carboxyl group attached to transfer-RNA (or activated in some other way) then joins with the amino group of the next amino acid in the sequence with the elimination of the valine-bound transfer-RNA. According to this picture, the growing peptide chain terminates at all stages in amino acyl-RNA.

The results reported here are equivalent to nonuniform labeling. That is, the residues of a particular amino acid in different portions of the chain have different specific activities. However, whether the "nonuniform labeling" observed by Steinberg *et al*[4] in studies of ovalbumin synthesis is due to the presence of incomplete protein chains in the ribosome remains to be determined. These authors do not exclude such a mechanism as an explanation of their findings. The proposed mechanism requires that the specific activity of a given amino acid *increase* along the chain from the N-terminal to the carboxyl-terminal end. Such a result has been reported recently for amylase synthesis.[22] However, interpretation of the data is complicated by the presence of unlabeled protein precursors of the enzyme. The results reported here do not conflict with those reported by Muir *et al*,[23] who found *uniform* labeling in hemoglobin. Their experiments were done *in vivo* and the mechanism proposed here would predict the attainment of uniform labeling after a small number of protein molecules were synthesized per ribosome. It is likely, therefore, that another explanation is needed for the nonuniform labeling observed by Kruh *et al*[24] after long term *in vivo* experiments. It should be noted that Shimura *et al*[25] found excess labeling in the N-terminal glycine of silk fibroin, indicating sequential synthesis from the carboxyl end of the chain.

Previously, we have concluded that incorporation of C^{14}-labeled amino acids into protein in this cell-free system represents mainly hemoglobin synthesis.[6] The presence of N-terminal C^{14}-valine in hemoglobin labeled in the cell-free system under favorable conditions supports this conclusion. The synthesis of the *complete* hemoglobin chains is limited, however, and the largest part of the C^{14}-amino acid incorporated in the cell-free systems represents synthesis of chains already started. That completed hemoglobin chains are formed is indicated by the experiments in which C^{14}-valine-labeled ribosomes incubated with C^{14}-valine in the cell-free system gave hemoglobin with the same percentage of N-terminal valine as did the intact cell.

We wish to thank Dr. James Bonner for his many helpful suggestions and his aid in the preparation of this manuscript.

*Present address: University of Kentucky Medical School, Lexington, Kentucky.

† This work was done during the tenure of an Established Investigatorship of the American Heart Association. These studies were supported by grants from the National Science Foundation and the American Heart Association.

[1] Hoagland M. B., in Proceedings of the 4th International Congress on Biochemistry, (New York: Pergamon Press, 1960), Vol. 8, p. 199. **8,** (1960).

[2] Rabinovitz, M., and M. E. Olson, *Exptl. Cell Research,* **10,** 747 (1956).

[3] Dintzis, H., H. Borsook, and J. Vinograd, in *Microsomal Particles and Protein Synthesis,* ed. R. B. Roberts (New York: Pergamon Press, 1958), p. 95.

[4] Steinberg, D., M. Vaughan, and C. Anfinsen, *Science,* **124,** 389 (1956).

[5] Loftfield R., *Proc. IVth International Cong. Biochem.,* **8,** 222 (1960).

[6] Schweet, R., H. Lamfrom, and E. Allen, these PROCEEDINGS, **44,** 1029 (1958).

[7] Morris, A., and R. Schweet, *Biochim. et Biophys. Acta,* submitted for publication.

[8] Schweet, R., and E. Allen, in preparation.

[9] Borsook, H., C. L. Deasy, A. J. Haagen-Smith, G. Keighley, and P. H. Lowy, *J. Biol. Chem.,* **196,** 669 (1952).

[10] Borsook, H., E. H. Fischer, and G. Keighley, *J. Biol. Chem.,* **229,** 1059 (1957).

[11] The abbreviations used are: Tris, tris (hydroxymethyl) aminomethane; TCA, trichloracetic acid; DNP, dinitrophenyl; PTH, phenylthiohydantoin.

[12] Allen, E., and R. Schweet, *Biochim. et Biophys. Acta,* **39,** 185 (1960).

[13] Sanger, F., *Biochem. J.,* **45,** 563 (1949).

[14] Blackburn, S., and A. Lowther, *Biochem. J.,* **48,** 126 (1951).

[15] Levy, A., *Nature,* **174,** 1216 (1954).

[16] Fraenkel-Conrat, H., J. Harris, and A. Levy, *Methods in Biochemical Analysis,* **2,** 359 (1954).

[17] Ts'o, P., J. Bonner, and H. Dintzis, unpublished data.

[18] Osawa, H., and K. Satake, *J. Biochem.* (Tokyo), **42,** 641 (1955).

[19] Kruh, J., and H. Borsook, *J. Biol. Chem.,* **220,** 905 (1956).

[20] The size of the incomplete hemoglobin chain is calculated for those ribosomes which complete and release hemoglobin. Approximately 25 per cent of the original radioactivity usually remains in the ribosomes after the cell-free incubation with C^{12}-amino acids. If these ribosomes contain smaller incomplete chains than those which complete and release their hemoglobin chains, the steady state size of the incomplete hemoglobin chain will be smaller.

[21] Loftfield, R., and E. Eigner, *J. Biol. Chem.,* **231,** 925 (1958).

[22] Yoshida, A., and T. Tobita, *Biochim. et Biophys. Acta,* **37,** 513 (1960).

[23] Muir, H., Neuberger, A., and J. Perrone, *Biochem. J.,* **52,** 87 (1952).

[24] Kruh, J., J. Dreyfus, and G. Schapira, *J. Biol. Chem.,* **235,** 1075 (1960).

[25] Shimura, K., H. Fukai, J. Sato, and R. Saeki, *J. Biochem., Japan,* **43,** 101 (1956).

---

*SPECIFIC ANTIBODIES TO THERMALLY DENATURED DEOXYRIBONUCLEIC ACID OF PHAGE T4**

BY L. LEVINE, W. T. MURAKAMI, H. VAN VUNAKIS, AND L. GROSSMAN

GRADUATE DEPARTMENT OF BIOCHEMISTRY, BRANDEIS UNIVERSITY

Communicated by A. D. Hershey, June 21, 1960

Immunochemical analyses[1] of antisera to ruptured T4 bacteriophage suggested that an antibody directed against deoxyribonucleic acid (DNA) might be present. In this paper we show that antibodies specific for DNA are indeed present in our antisera, and that they react chiefly with thermally denatured DNA.

Previous workers have reported immunological reactivity of native bacterial and mammalian DNA.[2–4]