(*8*). However, where his experimental arrangement included over the surface a layer of static air which provided a sufficient barrier to evaporation to give the erroneous conclusion that surface films were without effect (*1*), the current experiments permitted the liquid surfaces to be freely accessible to air currents and thus decreased the barrier.

No wholly satisfactory explanation of the action of adsorbed layers of protein to increase evaporation from their solutions is available. The fact that workers studying evaporation through spread monolayers of protein have not reported any increased evaporation, even when moving currents of air were employed (*9*), suggests that the situation may be different with monolayers. Enhanced evaporation appeared in Bull's work with continuously generated adsorbed layers or in the present work with newly formed absorbed films. In both cases the surface film was not in equilibrium with the bulk solution and protein was still adsorbing during evaporation. Where adsorption was virtually complete owing to long surface aging, or at higher protein concentrations where adsorption was completed more quickly, there was less enhancement of evaporation. This seems to imply that continuing adsorption of protein is related to the increase in evaporation rate, as was first suggested by Bull (*3*).

Globular protein molecules have a considerable amount of water associated with them, and the process of surface denaturation, in common with other types of denaturation, produces a decreased binding of water. Hence, adsorption of protein followed by denaturation at the surface could serve to transport a small excess of water to the surface region. However, as was pointed out (*3*), this amount of water is insufficient to account for the increased evaporation found.

In addition, Tovbin and Savinova (*10*) have investigated the nonsteady-state kinetics of water evaporation and have shown that at very short times of contact between water and a jet of moving air evaporation was enhanced. With increasing time of phase contact evaporation rates at first increased and then decreased, approaching steady-state values. In the present work the times of contact between moving air and the liquid surface, while impossible to assess, are nevertheless probably rather short (*11*).

LAYLIN K. JAMES, JR.
DONALD J. O. BERRY
Department of Chemistry, Lafayette College, Easton, Pennsylvania

References and Notes

1. V. K. LaMer, Ed., *The Retardation of Evaporation by Monolayers: Transport Processes* (Academic Press, New York, 1962).
2. B. V. Deryagin *et al.*, *Kolloidn. Zh.* **23**, 262 (1961).
3. H. B. Bull, *J. Biol. Chem.* **123**, 17 (1938).
4. ———, private communication.
5. I. Langmuir and V. J. Schaeffer, *J. Franklin Inst.* **235**, 119 (1943).
6. R. J. Archer and V. K. LaMer, *Ann. N.Y. Acad. Sci.* **58**, 807 (1954).
7. H. G. Houghton, *Physics* **4**, 419 (1933); N. A. Fuchs, *Evaporation and Growth of Drops in a Gaseous Medium* (Pergamon, New York, 1959).
8. G. Hedestrand, *J. Phys. Chem.* **28**, 1245 (1924).
9. F. Sebba and E. K. Rideal, *Trans. Faraday Soc.* **37**, 273 (1941).
10. M. V. Tovbin and E. V. Savinova, *Zh. Fiz. Khim.* **31**, 2445 (1957).
11. Supported, in part, by U.S. Public Health research grant A-4348(C1). We acknowledge the contribution of Frederick Churchill II, who confirmed the part played by the diffusional barrier to evaporation in his B.S. thesis, Lafayette College, 1962.

18 February 1963

Reticulocyte Protein Synthesis: Response of Ribosome Fractions to Polyuridylic Acid

Abstract. *Reticulocyte ribosomes with sedimentation coefficients greater than 100S ("heavy" ribosomes) appear to be considerably more active in hemoglobin synthesis than are 78S ribosomes. When assayed for the ability to synthesize polyphenylalanine in the presence of polyuridylic acid, the 78S ribosomes and "heavy" ribosomes have similar activities. Polyuridylic acid inhibits incorporation by "heavy" ribosomes of amino acids other than phenylalanine.*

Protein synthesis in rabbit reticulocytes occurs on "heavy" ribosomes that have a sedimentation coefficient greater than 100*S* (*1*). Ribosomes with a sedimentation coefficient of 78*S* comprise the major portion of the isolated ribosomes and do not appear to function in protein formation. Electron micrographs indicate that "heavy" ribosomes may be clusters of two or more 78*S* ribosomes (*2*). Polyuridylic acid (polyU), as well as other synthetic polyribonucleotides, can direct polypeptide synthesis on microsomes and ribosomes of a variety of mammalian cells (*3–5*). That this is true of ribosomes obtained from reticulocytes (*3, 5*) is of particular interest, since the formation of hemoglobin, which accounts for over 80 percent of the protein synthesized in these cells, is directed by a relatively stable form of RNA (*1, 6*). This study was undertaken to determine which class of reticulocyte ribosomes participates in the incorporation of phenylalanine into polyphenylalanine when this synthesis is directed by polyuridylic acid.

Reticulocytes were prepared from phenylhydrazine-treated rabbits (*7*). The cells were lysed either by two cycles of freezing and thawing (*3*) or by a method of "shock lysis" (*1*). The unlysed cells and cell debris were sedimented at 10,000*g* to yield a supernatant fraction (S-10) which was used directly for studies of the incorporation of amino acid and was also used to prepare ribosomes and a ribosome-free supernatant solution (*1*). Ribosomes were separated into fractions, differing in sedimentation coefficients, by sucrose density-gradient centrifugation (see legend, Fig. 1). The components of the cell-free system for amino acid incorporation (Fig. 1), the counting procedures for determining radioactivity, and the processing of samples for determining incorporation of amino acid into protein have been described (*3*). Two preparations of polyU (*8*) were used with similar results.

The distribution of the C^{14}-phenylalanine that was incorporated on ribosomes by an S-10 preparation, both in the absence (endogenous reaction) and presence of polyU, was analyzed by the sucrose density-gradient technique (Fig. 1). The peak in ultraviolet (UV) absorption is designated 78*S* (a figure based on data from an analytical ultracentrifuge) and represents the marker ribosomes. The UV-absorbing material and radioactivity to the right of the 78*S* peak represent lighter components (Fig. 1, peak *a*). In the absence of polyU, radioactivity was associated with ribosomal fractions which had sedimentation coefficients greater than 100*S*, and there was no appreciable radioactivity in the 78*S* region (Fig. 1*A*). On the other hand, when incorporation occurred in the presence of polyU, radioactivity was distributed throughout the different classes of ribosomes. It should be noted that ribosomes with sedimentation coefficients of about 110*S* (Fig. 1*B*, corresponding to tube 15) had approximately twice as many count/min per unit of optical density as ribosomes with sedimentation coefficients of 78*S*.

Different classes of ribosomes, separated by the sucrose density-gradient technique, were assayed for the incorporation of C^{14} phenylalanine both when polyU was and was not a constituent of the incorporating mixture (Fig. 2). In the absence of polyU the heavy ribosomes (pool III, Fig. 2) incorporated greater amounts of phenylalanine. In contrast, when polyU was present, the amount of phenylalanine incorporated was about the same regardless of the size of the ribosomes. Additional studies indicated that incorporation was about equal for "light" or "heavy" ribosomes when the concentration of polyU was only 25 μg/0.3 ml or when a synthetic polyribonucleotide containing both uridylic and cytidylic acid in a ratio of 5 to 1 was present.

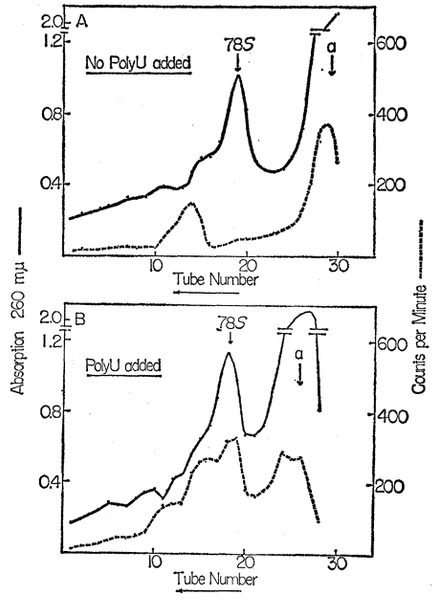

Fig. 1. The association of C^{14}-L-phenylalanine with sucrose gradient fractions of reticulocyte ribosomes after incorporation in the absence and presence of polyU. The complete reaction mixture had a total volume of 1 ml and contained the following components (in μmoles/ml unless otherwise specified): tris-HCl buffer, *p*H 7.6; magnesium acetate, 5.0; KCl, 46; mercaptoethanol, 4.3; adenosine triphosphate, 1.0; phosphoenolpyruvate (PEP), 4.0; guanosine triphosphate, 0.03; cytidine triphosphate, 0.03; uridine triphosphate, 0.03; phosphoenolpyruvate kinase, 40 μg; C^{14}-L-phenylalanine, 2.7 mμmole, specific activity 160 mc/μmole (about 233 $\times$ 10^3 count/min); a mixture of C^{12}-L-amino acids (*3*), excluding phenylalanine, 0.04 of each; and the equivalent of 30 mg of S-10 protein. Samples were incubated in the absence and presence of polyU (330 μg/ml) at 37°C for 40 min. After chilling at 4°C, 1 mg of carrier reticulocyte ribosomes was added to the incubation mixture and 0.7 ml of each sample was then directly layered on a sucrose gradient. The latter was prepared with solutions of 5 percent and 20 percent sucrose containing 1.5 $\times$ 10^{-3}*M* $MgCl_2$, 5 $\times$ 10^{-2}*M* KCl, and 10^{-3}*M* tris-HCl buffer, *p*H 7.5. Centrifugation was at 25,000 rev/min in the Spinco SW-25 rotor at 4°C for 2.5 hours. Arrows under tube numbers indicate direction of sedimentation. Fractions of the gradients were collected and analyzed (*1*). Samples processed for total C^{14}-phenylalanine incorporation indicated a fivefold stimulation by polyU in this particular experiment. The UV-absorbing material and radioactive material sedimenting in region "*a*" includes hemoglobin as indicated by the absorption spectrum.

Thus, in addition to acting on 78*S* ribosomes, polyU can act on the "heavy" ribosomes, which participate in hemoglobin synthesis. It was of interest to determine whether in so doing, polyU inhibits the endogenous reaction. Since polyU specifically directs the incorporation of phenylalanine in bacterial as well as in mammalian systems (*3, 4*), inhibition of the endogenous reaction could be detected as the inhibition of incorporation of amino acids other than phenylalanine. That polyU caused an eightfold increase in the incorporation of C^{14} phenylalanine by the S-10 fraction of reticulocytes while it inhibited the incorporation of nine other amino acids is shown in Table 1. The mean percentage inhibition was 52.5, with a range of 42.9 to 68.8.

The effect of increasing concentrations of polyU on the incorporation of C^{14}-L-leucine and C^{14}-L-phenylalanine in a cell-free system containing only "heavy" ribosomes (Fig. 3) was studied. Maximum inhibition of incorporation of leucine occurred with 10 μg of polyU. Despite higher concentrations of polyU, incorporation of leucine remained at approximately 67 percent of that obtained in the absence of polyU. On the other hand, approximately 50 μg of polyU was required to produce the maximum incorporation of phenylalanine. With 10 μg of polyU, incorporation of phenylalanine was less than that obtained in the absence of polyU. By contrast, the addition of increasing amounts of polyU to a system that contained predominantly 78*S* ribosomes, which had negligible endogenous activity, did not reveal a zone where the incorporation of phenylalanine was inhibited. This result suggests that there is a concentration of polyU at which the synthesis of polyphenylalanine is not sufficient to compensate for the inhibition of endogenous incorporation of phenylalanine.

Table 1. Inhibition by polyU of the endogenous incorporation of amino acids by a cell-free extract of rabbit reticulocytes *

C^{14}-L-amino acids	Radioactivity incorporated (count/min)		Inhibition by polyU (%) †
	No polyU	100 μg polyU	
Phenylalanine	487	3684	
Leucine	1491	721	51.6
Valine	354	164	53.6
Tyrosine	203	118	42.9
Lysine	138	66	52.2
Isoleucine	116	65	44.0
Serine	93	29	68.8
Arginine	69	34	50.7
Alanine	66	27	59.0
Proline	40	20	50.0

* Reaction mixtures were similar to those for Fig. 1. C^{14}-L-amino acids (specific activity 850 to 1000 μc/mg) were tested individually in the presence of a mixture of C^{12}-amino acids minus the particular C^{14}-amino acid tested. Reaction mixtures were incubated and processed as for Fig. 2. † Percentage inhibition represents the ratio of count/min incorporated in the presence of polyU to count/min incorporated in the absence of polyU.

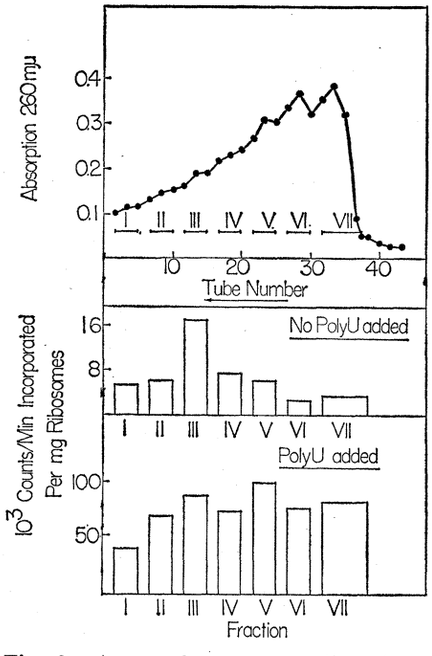

Fig. 2. Assay of sucrose gradient fractions for their ability to participate in endogenous and polyU-directed incorporation of C^{14}-phenylalanine. Thirty milligrams of ribosomes were isolated from a "shock lysate" of rabbit reticulocytes and fractionated through a sucrose gradient (legend, Fig. 1). Fractions were pooled and 0.4 mg of each fraction was assayed for C^{14}-phenylalanine incorporation in the absence and presence of polyU (330 μg/ml). The total volume of the assay system was 0.3 ml and, in addition to the ribosome fractions being tested, it contained the ribosomal supernatant fraction in an amount equivalent to 3 mg protein. The remaining components are as given in Table 1. Reaction mixtures were incubated at 37°C for 40 min and processed for total C^{14} incorporation into protein (*3*). The results are expressed as count/min incorporated per milligrams of ribosomes added.

The present study indicates that in the presence of polyU, 78*S* reticulocyte ribosomes, as well as heavier ribosomes, incorporate C^{14}-phenylalanine with approximately equal activity, whereas in the absence of polyU only the "heavy" ribosomes incorporate phenylalanine. If polyU, which appears to serve as a synthetic messenger (*9*), is supplied to 78*S* ribosomes they can then participate in polypeptide synthesis.

These observations are in general accord with studies of *Escherichia coli* ribosomes (*10–14*). In extracts of *E. coli*, protein synthesis is confined to an "active" fraction of ribosomes with a sedimentation coefficient of 100*S*; this fraction comprises less than 10 percent of all the ribosomes in cell-free preparations (*10*). Evidence has been presented that "messenger" RNA is selectively attached to, or incorporated into, the "active" ribosomes (*11, 14, 15*). Recent studies indicate that polyU causes the conversion of 70*S* *E. coli* ribosomes to 100*S* to 200*S* ribosomes, and that it is these "heavy" ribosomes which participate in polyphenylalanine synthesis (*12–14*). Further studies are required to determine whether polyU acts similarly with 78*S* reticulocyte ribosomes.

Our data indicate that polyU can function with the same fraction of ribosomes which engages in hemoglobin synthesis. The fact that polyU inhibits the incorporation of amino acids other than phenylalanine suggests that it can inhibit the synthesis of hemoglobin by heavy ribosomes.

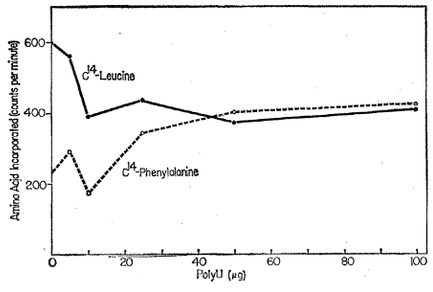

Fig. 3. A comparison of the effect of increasing concentrations of polyU on the incorporation of C^{14}-phenylalanine and C^{14}-leucine by "heavy" ribosomes. Except for the presence of either C^{14}-leucine or C^{14}-phenylalanine and the appropriate mixture of C^{12}-amino acids, the reaction mixtures were similar to those indicated in the legend for Fig. 2. PolyU was added at the concentrations shown. Samples were incubated and processed as described for Fig. 2.

This suggests that the pattern of protein synthesis by ribosomes from highly differentiated animal cells may be redirected by new templates (*3*). It remains to be determined whether or not polyU actually causes a physical displacement from the heavy ribosomes of an endogenous messenger (*16*).

I. BERNARD WEINSTEIN
ALAN N. SCHECHTER
EDWARD R. BURKA, PAUL A. MARKS
Department of Medicine, Columbia University College of Physicians and Surgeons, New York 32

References and Notes

1. P. A. Marks, E. R. Burka, D. Schlessinger, *Proc. Natl. Acad. Sci. U.S.* **48**, 2163 (1962).
2. J. R. Warner, A. Rich, C. E. Hall, *Science* **138**, 1399 (1962).
3. I. B. Weinstein and A. N. Schechter, *Proc. Natl. Acad. Sci. U.S.* **48**, 1686 (1962).
4. E. Maxwell, *ibid.* **48**, 1639 (1962).
5. H. R. V. Arnstein, R. A. Cox, J. A. Hunt, *Nature* **194**, 1042 (1962).
6. H. Borsook and K. Simura, personal communication; P. A. Marks, C. Willson, J. Kruh, F. Gros, *Biochem. Biophys. Res. Commun.* **8**, 9 (1962).
7. H. Borsook, C. L. Deasey, A. J. Haagen-Smit, G. Keighley, O. H. Lowry, *J. Biol. Chem.* **196**, 669 (1952).
8. One preparation was supplied by Dr. J. Fresco of Princeton University and the other was obtained from Miles Laboratories.
9. M. W. Nirenberg and J. H. Matthaei, *Proc. Natl. Acad. Sci. U.S.* **47**, 1588 (196 P. Lengyel, J. F. Speyer, S. Ochoa, *ibid.* **47**, 1936 (1961).
10. A. Tissières, D. Schlessinger, F. Gros, *ibid.* **46**, 1450 (1960).
11. R. W. Risebrough, A. Tissières, J. D. Watson, *ibid.* **48**, 430 (1962).
12. G. J. Spyrides and F. Lipman, *ibid.* **48**, 1977 (1962).
13. S. H. Barondes and M. W. Nirenberg, *Science* **138**, 813 (1962).
14. W. Gilbert, *J. Mol. Biol.*, in press.
15. F. Gros *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **26**, 11 (1961).

16. Supported by grants CY 2332 and GM 07368 of the U.S. Public Health Service. One of us (I.B.W.) is a career scientist of the Health Research Council of the City of New York, I-190; another (E.R.B.) is a trainee in hematology by grant 2A-5231 from the U.S. Public Health Service.

11 January 1963

Histone and DNA in Isolated Nuclei from Chicken Brain, Liver, and Erythrocytes

Abstract. *In isolated brain and liver nuclei the ratio of histone to deoxyribonucleic acid is lower than in nuclei from erthrocytes. Deoxyribonucleoproteins from brain and liver, in contrast to deoxyribonucleoproteins from erythrocytes, are more soluble in isotonic sodium chloride.*

An understanding of the mechanisms which regulate gene activity in higher organisms requires a knowledge of the structure and function of the molecular complexes of deoxyribonucleic acid (DNA) in the cell nucleus. Since tissue differentiation is the result of differential expression of genetic endowment, comparative investigation of the molecular complexes of DNA in different tissues may yield some information on the problem of gene regulation. Whereas histones have previously been implicated in the regulation of gene activity (*1*), the evidence for tissue specificity of histones is still controversial (*2*). Therefore, in our study of deoxyribonucleoproteins (DNP) we have attempted to determine if there are quantitative differences in the ratio of histone to DNA in brain, liver, and erythrocyte nuclei of the adult chicken.

Tissues from 1-year-old New Hampshire Red hens were used in all cases. All procedures were performed at 0° to 4°C unless otherwise specified. Highly pure nuclei from brain and liver were isolated by methods described previously for rat tissues (*3*), except that adenosine triphosphate was omitted from the sucrose medium in the final purification step. Erythrocyte nuclei were obtained as follows: blood was collected in 20 volumes of cold 0.1 m*M* $KHCO_3$, and after allowing 10 minutes for complete hemolysis, the lysed cells were spun down at 1000*g* (av.) for 10 minutes and then homogenized (with a very tight pestle in a Dounce homogenizer to remove stroma adherent to the nuclei) in 20 volumes of the same sucrose homogenizing medium used for brain and liver. This homogenate was then treated in the same way the brain and liver homogenates were treated. The yield of nuclei was approximately 10 percent for brain, 40 percent for liver, and 40 percent for erythrocytes.

Histone is defined, in this report, as that protein in the isolated cell nucleus which, in addition to being soluble in 2*M* NaCl, at *p*H 1, also retains its solubility in water after being precipitated in 25 percent trichloroacetic acid (TCA) containing 1*M* NaCl. Calf thymus histone (Worthington lot H-562), which had been prepared (*4*) by washing homogenized thymus three times with isotonic saline, extracting the residue with 1*M* NaCl, precipitating the