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.

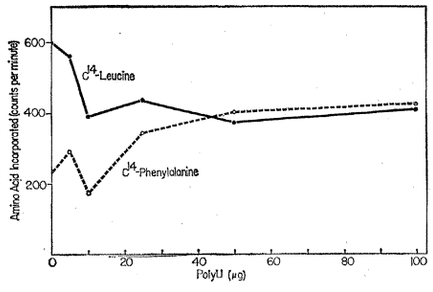

Fig. 3. A comparison of the effect of increasing concentrations of polyU on the incorporation of C^{14}-phenylalanine and C^{14}-leucine by "heavy" ribosomes. Except for the presence of either C^{14}-leucine or C^{14}-phenylalanine and the appropriate mixture of C^{12}-amino acids, the reaction mixtures 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*).

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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 (1961); 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.

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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

extracted DNP by dilution with water, re-extracting the DNP precipitate with 0.2*M* HCl, and finally re-precipitating the histone extract with 4 volumes of ethanol at *p*H 10.3, satisfied this definition entirely. When this histone preparation was dissolved in 2*M* NaCl containing 0.1*M* HCl, it was quantitatively precipitated, at final concentrations as low as 25 μg/ml, on addition of an equal volume of 50 percent trichloroacetic acid. The mixture of histone and trichloroacetic acid was allowed to stand 1 hour and then centrifuged for 45 minutes at 100,000*g* (av.) to ensure quantitative recovery of histone. Such histone precipitates were readily soluble in water at room temperature.

Isolated nuclei were extracted for 18 to 42 hours with sufficient 2*M* NaCl to give a final concentration of DNA-phosphorus of 0.3 to 0.8 μmole/ml. The extraction was aided by gentle agitation with a stirring rod; vigorous stirring, which produces extensive foaming, results in a very low yield of histone, presumably because of surface denaturation (*5*). Hydrochloric acid was then carefully added, with stirring, to a final concentration of 0.1 or 0.2*M* to achieve complete extraction of histone and quantitative precipitation of DNA. The mixture was stirred for 1 hour and centrifuged for 30 minutes at 1500*g* (av.), and the supernatant was saved. The precipitate was washed once with a small amount of 2*M* NaCl containing the appropriate concentration of HCl, and the wash was combined with the previous supernatant, whereas the precipitate was saved for DNA determination. Two milliliters of 50 percent trichloroacetic acid were added to 2 ml samples of the supernatant fraction, and the mixture was allowed to stand for 1 hour and then was centrifuged for 45 minutes at 100,000*g* (av.) in the Spinco SW 39 rotor. The drained, finely divided precipitate was extracted by stirring with 2 ml of water for 1 hour at room temperature. The extract was then centrifuged for 30 minutes at 1500*g* (av.) to remove the water-insoluble residue, and the protein content of the supernatant was measured (*6*).

The HCl precipitate, containing the DNA and other acid-insoluble components of the nucleus, was washed once with 0.2*M* perchloric acid, once with ethanol in the cold, once with ethanol at room temperature, twice with a mixture of chloroform and methanol (1:1 by volume) at room temperature, once more with ethanol at room temperature, and finally with 0.2*M* perchloric acid. All washes were discarded. The final precipitate was extracted twice with 0.5*M* perchloric acid at 70°C for 15 minutes, and the DNA content of the pooled extracts was measured (*7*).

Table 1. Ratio of histone to DNA-phosphorus in chicken brain, liver, and erythrocyte nuclei, determined under different conditions of extraction. Extraction was done with 2*M* NaCl. [H⁺] was used to precipitate DNA.

Extraction time (hr)	[H⁺] (*M*)	Ratio of histone to DNA-phosphorus (μg/μmole)		
		Brain	Liver	Erythrocyte
		Experiment 1		
18	0.1	250	277	306
		Experiment 2		
18	0.1	280	262	326
		*Experiment 3**		
18	0.1	254	282	316
		Experiment 4		
18	0.2		256	326
		Experiment 5		
42	0.1		295	
		Experiment 6		
42	0.2	282	296	351
Mean ± S.D.†		266±17	278±16	325±17

* The initial tissue homogenates were allowed to stand at 0°C for 90 minutes before their first centrifugation. † Standard deviation, S.D.

Crystalline bovine pancreatic ribonuclease (Worthington) was used as a protein standard; a solution with an extinction of 0.715 at 277.5 mμ in 0.01*M* potassium phosphate, *p*H 6.8, was assumed to contain 1 mg/ml (*8*). Calf thymus DNA (Worthington) was used as a DNA standard; a solution of native DNA with an extinction of 6600 at 260 mμ in 0.01*M* potassium phosphate, *p*H 6.8, was assumed to be 1*M* in DNA-phosphorus (*9*). All colorimetric analyses were performed in duplicate. In each experiment the preparation and all subsequent analyses of the three types of nuclei were done simultaneously (Table 1).

The ratios of histone to DNA found in isolated nuclei from brain, liver, and erythrocytes are presented in Table 1. Also set forth in the table are the various conditions of extraction employed in an attempt to assess artifacts contributed by: (i) possible proteolysis or DNA degradation during the procedure, (ii) time of extraction with 2*M* NaCl; or (iii) strength of acid used to precipitate DNA and to extract histone. In all cases erythrocyte nuclei have a definitely higher histone content than brain or liver nuclei. On the basis of the *t* test, differences between mean values for brain and erythrocyte, as well as liver and erythrocyte, are statistically significant ($p < .01$). Different procedures used for separating histone from DNA did not, in general, significantly change the ratio of histone to DNA for a given tissue. This suggests that artifacts, such as proteolysis, do not account for the differences observed between tissues. However, in liver and erythrocyte, 42-hour extraction gave slightly higher values for the ratio of histone to DNA, but the difference between these two tissues remained. The mean ratio of 325 found for the erythrocytes corresponds to approximately equal amounts of protein and DNA by weight. This is comparable to the ratio of protein to DNA found for calf thymus DNP (*10*).

Ribonuclease is a crystalline reagent whose concentration can be readily and accurately determined spectrophotometrically; therefore we used it rather than histone as the protein standard and, as a result, our values may not be precisely equal to those which would have been obtained with histone. The variations found between tissues are independent of this factor.

The solubility of calf thymus DNP depends on ionic strength. When sheared, this DNP is soluble in solutions whose salt concentration is less than 0.01*M* and more than 0.7*M*, but it is relatively insoluble in the range between these two values (*9, 10*). Sheared calf thymus DNA, in contrast, is soluble over this entire range. It is conceivable then that variations in the ratio of protein to DNA in DNP would cause variations in the dependence of solubility of DNP on ionic strength. Therefore, in an alternative approach to the question of the existence of quantitative differences in the ratio of histone to DNA in various tissues, we have investigated: (i) the ability of a hypotonic medium to solubilize DNP which has been subjected to shear in homogenates of brain, liver, and erythrocytes, and (ii) the ability of 0.15*M* NaCl to precipitate this solubilized DNP. We found that if brain, liver, or hemolyzed erythrocytes were thoroughly homogenized with a tight pestle in a Dounce homogenizer in 60 volumes of 0.1 m*M* $KHCO_3$, *p*H 6.8, and then centrifuged for 30 minutes at 1500*g* (av.), less than 15 percent of the erythrocyte

DNP (determined as DNA) of the original homogenate remained in the supernatant, whereas over 50 percent of the brain or liver DNP remained in the supernatant. If supernatants from the three tissues containing approximately equal concentrations of DNA (0.02 to 0.04 μmole of DNA-phosphorus per milliliter) were then made 0.15*M* in NaCl, let stand for 30 minutes, and then centrifuged for 30 minutes at 1500*g* (av.), over 95 percent of the erythrocyte DNP was precipitated, but less than 50 percent of the brain and liver DNP was precipitated. Erythrocyte DNP thus behaves like calf thymus DNP since it is highly insoluble in isotonic salt solutions.

Similar solutions of brain and liver DNP's, on the other hand, are not completely precipitated by isotonic salt, which is what one might expect of a set of DNA molecules that are less completely complexed with histones. It is conceivable that protease or deoxyribonuclease activity in the brain and liver homogenates prevented the DNP in these two tissues from sedimenting as easily in isotonic salt; however, an experiment in which the initial brain, liver, and erythrocyte homogenates were allowed to stand for 2 hours before their first centrifugation yielded essentially the same results. It must be emphasized that the above experiments were performed on tissue homogenates and thus alterations in the precipitability of DNP caused by other tissue proteins could not be completely controlled, but the highly dilute homogenates used should minimize these possibilities.

If we assume that the protein measured in the first set of experiments is actually part of the native DNP complex, then the results of both types of experiments would indicate that there may be different amounts of protein associated with the DNA of different tissues. If again we assume that the histone associated with DNA functions as an inhibitor of gene activity (*1*), then our results may be interpreted to suggest that the DNA's of brain and liver direct the synthesis of a greater variety of proteins than does the DNA of the erythrocyte; however, these interpretations are largely speculative, for it must be kept in mind that substances other than histone may play a role in the regulation of gene activity (*11*).

Although attempts were made to minimize and control for possible protease and DNase activity, it cannot be conclusively proved that they are not factors in our results. Further validation might be obtained by isolation and characterization of the native, undegraded DNP complex from brain, liver, and erythrocyte, although here, too, ultimate proof that the isolated material is truly native and undegraded will be difficult. There is a need for improved methods for isolating undegraded DNP's from a wide variety of tissues, in order to study this complex both structurally and functionally. Our results suggest that methods developed for one tissue are not necessarily directly applicable to another if one wishes to isolate a representative portion of the chromosomes from a given tissue (*12*).

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References and Notes

1. E. Stedman and E. Stedman, *Nature* **166**, 780 (195(→ R. C. Huang and J. Bonner, *Proc. Natl. Acad. Sci. U.S.* **48**, 1216 (1962).
2. L. Hnilica, E. W. Johns, J. A. V. Butler, *Biochem. J.* **82**, 123 (1962); J. A. V. Butler, *J. Gen. Physiol.* **45**, Supplement, 195 (1962).
→ M. B. Sporn, T. Wanko, C. W. Dingman, *J. Cell Biol.* **15**, 109 (1962); S. H. Barondes, C. W. Dingman, M. B. Sporn, *Nature* **196**, 145 (1962).
4. Dr. Richard Egan, Worthington Biochemical Corp., Freehold, N.J., personal communication.
5. P. F. Davison and J. A. V. Butler, *Biochim. Biophys. Acta* **21**, 568 (1956).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
7. K. Burton, *Biochem. J.* **62**, 315 (1956).
8. M. Sela and C. B. Anfinsen, *Biochim. Biophys. Acta* **24**, 229 (1957).
9. E. Chargaff, in *The Nucleic Acids*, E. Chargaff and J. N. Davidson, Eds. (Academic Press, New York, 1955), vol. 1, p. 336.
10. G. Zubay and P. Doty, *J. Mol. Biol.* **1**, 1 (1959).
→ G. S. Stent and S. Brenner, *Proc. Natl. Acad. Sci. U.S.* **47**, 2005 (1961); C. G. Kurland and O. Maaloe, *J. Mol. Biol.* **4**, 193 (1962); K. Paigen, *J. Theoret. Biol.* **3**, 268 (1962).
12. We thank Dr. Donald B. Tower for his support and encouragement of this work.

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Errorless Discrimination Learning in the Pigeon: Effects of Chlorpromazine and Imipramine

Abstract. *Chlorpromazine or imipramine disrupts a pigeon's performance on a discrimination between a vertical and horizontal line only if the discrimination was learned with errors. Errorless learning is obtained if training starts with an easy-to-learn discrimination of color and shifts progressively to the more difficult horizontal-vertical discrimination.*

Recent experiments (*1*) have shown that a pigeon is able to acquire a discrimination of color and the orientation of a line without any "errors." An "error" is the failure to respond to the stimulus correlated with reinforcement (S+) or a response to the stimulus correlated with nonreinforcement (S−). Errorless learning is accomplished by starting discrimination training immediately after the response to S+ has been conditioned, and by progressively reducing the difference between S+ and S− from an initially large value to the relatively smaller final value.

When a discrimination is learned without errors, certain characteristics of performance, normally observed in discrimination performance after learning with errors, are lacking (*1*). These are (i) an increase in the rate (or decrease in the latency) of the response to S+, (ii) sporadic bursts of responses to S−, separated by long intervals of no responses to S−, and (iii) "emotional" responses to S−. The present study investigates another frequently observed characteristic of discrimination performance, the disruption of performance that follows the administration of certain drugs (*2*). Specifically, the effects of chlorpromazine and imipramine were studied after discrimination learning by the pigeon with, and without, errors. These drugs disrupt discrimination performance in the pigeon (*3*).

Discrimination training was carried out in a standard operant conditioning apparatus (*1*). The discriminative stimuli were projected on the response key during discrete, automatically programmed trials. Each trial was terminated by a single response or by the failure to respond within 5 seconds of the onset of the trial. A response that occurred during an S+ trial was immediately reinforced. Between trials, the "house light" remained on but the key was dark for intervals (mean length 30 seconds). S+ and S− trials alternated in random succession, unless an error was made, in which case the trial was repeated.

The subjects were four White Carneau male pigeons with no prior experimental history. Two pigeons (Nos.