than is eclosion in *D. persimilis* (the less arid species). The theory is that the wings of flies eclosing when humidity is low are likely to dry before straightening. Flies with abnormal wings are not as motile, nor are they as effective once they locate a mate (*12*). Flies cultured in the laboratory do not experience desiccation after dawn (that is, when lights are on) because the stoppered bottles maintain high humidities continuously. The absence of humidity cycles in laboratory cultures can account for only part of the increased variability of the Ore-R strain. In natural conditions humidity is relatively high for some time preceding dawn; therefore, humidity cycles would not account for the difference between early eclosion strains of the laboratory and wild populations.

DALE L. CLAYTON
JOHN V. PAIETTA
Department of Biology,
Walla Walla College,
College Place, Washington 99324

References and Notes

1. C. S. Pittendrigh, in *Circadian Clocks*, J. Aschoff, Ed. (North-Holland, Amsterdam, 1965), pp. 277–297.
2. J. Aschoff, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 11 (1960).
3. K. S. Rawson, *ibid.*, p. 105; C. S. Pittendrigh, and V. G. Bruce, in *Photoperiodism and Related Phenomena in Plants and Animals*, R. B. Withrow, Ed. (AAAS, Washington, D.C., 1959), pp. 475–505.
4. F. Halberg, in *Photoperiodism and Related Phenomena in Plants and Animals*, R. B. Withrow, Ed. (AAAS, Washington, D.C., 1959), pp. 803–878; F. Halberg, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 289 (1960).
5. Differences also exist—for example, the apparent absence of diurnal rhythms in cave organisms [O. Park, T. W. Roberts, S. J. Harris, *Amer. Natur.* **45**, 154 (1941)].
6. E. Bunning, *Jahrb. Wiss. Bot.* **77**, 283 (1932).
7. A. S. Danilevskii, *Entomol. Oborzr.* **36**, 5 (1957).
8. R. J. Konopka and S. Benzer, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 2112 (1971).
9. R. Enns, personal communication.
10. R. C. Roberts, in *Behavior-Genetics Analysis*, J. Hirsch, Ed. (McGraw-Hill, New York, 1967), pp. 214–257.
11. C. S. Pittendrigh, in *Behavior and Evolution*, A. Roe and G. G. Simpson, Eds. (Yale Univ. Press, New Haven, Conn., 1958), pp. 390–416.
12. A. G. Bennet-Clark and A. W. Ewing, *Nature* **215**, 669 (1967).
13. Unfortunately, thermostat failure on 14 May 1972 destroyed these lines in generations 13 and 17, respectively.

17 July 1972

S-100 Protein Synthesis by Isolated Polyribosomes from Rat Brain

Abstract. *The radioactive proteins synthesized in a cell-free system and released from polyribosomes from rat brain were analyzed for the presence of a protein found only in the nervous system. Polyribosomes from rat liver were also studied to demonstrate the organ specificity of the radioactive product synthesized in vitro. The S-100 protein constituted 0.15 percent of the radioactive proteins released from brain polyribosomes.*

The S-100 protein, an acidic cytoplasmic protein, is found only in the nervous system (*1*). Although its role in neural function is not understood, it provides an organ-specific marker to study macromolecular synthesis in in vivo and in vitro neural systems. Studies of rat brain with in vivo labeling suggested that S-100 had high rates of synthesis and degradation (*2*). However, a reexamination of the turnover rate of S-100 protein in the whole brain of the rat indicated that the degradation of this protein in vivo occurs at about the same rate as that of total soluble protein (*3*). The degradation rates of S-100 and total protein were also similar in clonal lines of glial cells in culture. The measured rate of S-100 protein synthesis in culture was only a fraction of 1 percent of the total synthesized protein (*4*). Previous studies with microsomal or ribosomal preparations from rabbit brain indicated that 15 percent of the labeled, released protein could be precipitated with antiserum to S-100 protein (*5*). Because of these conflicting reports concerning synthesis and degradation, we reinvestigated S-100 protein synthesis in cell-free preparations with rat brain polyribosomes. Our results indicate that only a small percentage of the protein released from polyribosomes is S-100 protein.

Brains and livers from Sprague-Dawley rats were placed into ice-cold 0.25*M* sucrose–NH_4Cl buffer [0.1*M* NH_4Cl, 0.03*M* tris(hydroxymethyl)aminomethane (tris)-HCl, *p*H 7.4, 0.005*M* $MgCl_2$, and 0.005*M* β-mercaptoethanol (B-ME)]. Both tissues were minced, were rinsed several times, and were homogenized with eight to ten strokes of a Teflon and glass homogenizer. The homogenate was centrifuged for 10 minutes at 27,000*g*, and the resulting solution was centrifuged for 90 minutes at 195,000*g*. The supernatant solution from the second centrifugation was placed on a Sephadex G-25 column equilibrated with NH_4Cl buffer. This high-speed supernatant (HSS) was used as a source of enzymes and transfer RNA. To prepare polyribosomes, we rehomogenized the pellet centrifuged at 27,000*g* and centrifuged it again. The resulting supernatant solution was combined with the polyribosomes in the pellet from the 195,000*g* centrifugation. After suspension, the polyribosome solutions were treated with a mixture of sodium deoxycholate (DOC) and Tween 40; the final concentration of the solutions was 1.0 percent DOC, 1 percent Tween with brain polyribosomes, and 1.3 percent DOC, 1 percent Tween with liver polyribosomes. The polyribosomes treated with detergent were kept at 4°C for 10 minutes, and then centrifuged at 27,000*g* for 10 minutes. The supernatant solutions were then layered on 0.5*M* to 2.0*M* discontinuous sucrose gradients (in NH_4Cl buffer), and were centrifuged at 4°C for 24 hours at 130,000*g*. The polyribosome pellets were then rinsed with 0.25*M* sucrose–NH_4Cl buffer, suspended by mild homogenization in the same buffer, and centrifuged at 1000*g* for 10 minutes. The polyribosomes in the supernatant were stored at −70°C until needed.

The cell-free reaction mixture (1.0 ml) to study protein synthesis contained: 100 μmole of NH_4Cl, 30 μmole of tris-HCl, *p*H 7.4, 5 μmole of $MgCl_2$, 5 μmole of B-ME, 1 μmole of adenosine triphosphate (ATP), 1 μmole of guanosine triphosphate, 10 μmole of creatine phosphate, 4 enzyme units of creatine phosphokinase (Sigma), 20 nmole each of 15 amino acids in a mixture, and 20 μc each (*6*) of [^{3}H]leucine, [^{3}H]lysine, [^{3}H]phenylalanine, and [^{3}H]valine. In addition, each reaction contained 6 absorbancy units (at 260 nm) of polyribosomes, and 1.2 absorbancy units (at 280 nm) of HSS. Reaction mixtures were incubated for 60 minutes at 37°C, then chilled to 4°C, and centrifuged for 2 hours at 280,000*g* to separate the released proteins from the polyribosomes.

The amount of S-100 protein released from polyribosomes was determined by a modification of the method of Herschman (*4*). Suitable samples of released protein were placed on Sephadex G-15 columns [equilibrated in a solution of 0.01*M* B-ME and phosphate-buffered saline (PBS) (*7*)] to remove unincorporated amino acids and NH_4Cl.

Table 1. The amount of S-100 protein in total protein released by polyribosomes from rat brain and liver. Polyribosomes were isolated from 35-day-old animals, and the amount of S-100 protein was determined as described in the text. The amount of radioactivity in the proteins was determined by the method of Mans and Novelli (*9*). Counting efficiencies were in the range of 33 to 40 percent for all samples.

Sample	Radioactivity (count/min)			
	Experiment 1		Experiment 2	
	Brain	Liver	Brain	Liver
Polyribosome-free supernatant	840,000	860,000	700,000	680,000
After Sephadex G-15	595,000	580,000	560,000	540,000
After Diaflo concentration	350,000	350,000	325,000	300,000
After heat and centrifugation	220,000	95,000	250,000	100,000
After Sephadex G-25	215,000	95,000	220,000	100,000
S-100 protein fraction of gel	1,890	600	1,170	130
S-100 protein in brain*	1,290	0	1,040	0
S-100 protein (%)†	0.15	0	0.15	0

* Determined by subtracting the radioactivity in protein released by the liver polyribosomes from that in protein released by the brain polyribosomes. † Radioactivity of S-100 protein/radioactivity in polyribosome-free supernatant.

The excluded volume, as determined with dextran blue, was concentrated to 2.0 ml with a Diaflo UM-2 membrane (molecular weight exclusion = 1000; Amicon). Samples were heated to 60°C for 10 minutes, and then centrifuged for 10 minutes at 27,000*g*. The supernatants were placed on Sephadex G-25 in PBS. The antibody precipitation and sodium dodecyl sulfate (SDS) polyacrylamide gel separation of S-100 protein has been described (*4*). Briefly, the labeled S-100 protein was precipitated with nonradioactive carrier S-100 protein (23 μg) and specific antiserum (0.1 ml). The pellets were incubated for 1 hour at 37°C and overnight at 4°C, were washed three times with PBS, and then dissolved in 0.05 ml of buffer containing SDS and B-ME for 3 hours at 37°C. The resulting solutions were subjected to electrophoresis on 10 percent acrylamide gels containing SDS. Gels were cut into 1-mm slices, and were counted in an Isocap 300 counter (Nuclear-Chicago).

A profile of the radioactivity in the polyacrylamide gels of precipitates of S-100 protein (prepared from the protein released by polyribosomes from rat brain and liver) and antiserum to the protein is shown in Fig. 1. The fastest moving fraction in the brain sample contained the labeled S-100 protein. The liver sample also contained a small amount of radioactivity in the fastest migrating fraction. This radioactivity probably represents proteins trapped in the antibody-antigen complex, and released during treatment with SDS. The fact that the liver sample did contain some radioactivity in the area of migration of the S-100 protein emphasizes the importance of using nonneural tissues as controls. The radioactive profile of polyribosomal proteins from the brain, which were precipitated with antibody to S-100 protein is similar to those obtained from tissue culture of rat glial cells (*4*).

The results of two experiments designed to measure the amount of S-100 protein released by polyribosomes are shown in Table 1. Both experiments started with 5 to 10 ml (about 800,000 count/min) of protein released by polyribosomes. After chromatography on Sephadex G-15 and concentration with a Diaflo membrane, the samples contained about 300,000 count/min each. When the samples were heated to 60°C, about one-third of the brain protein and two-thirds of the liver protein were lost. The S-100 protein, in the presence of B-ME, is not denatured by the heat. In these experiments, 0.15 percent of the protein released by polyribosomes was S-100 protein.

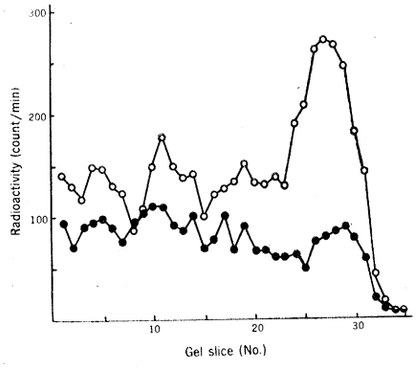

Fig. 1. Polyacrylamide gel patterns of precipitates of S-100 protein (prepared from proteins released from polyribosomes from rat brain and liver) and antibody to S-100 protein. The proteins released from polyribosomes from 35-day-old animals were analyzed as described in the text, and as shown in experiment 1, Table 1. The solubilized antigen-antibody precipitates were applied at slice number 1. Slices 23 to 32 contain S-100 protein as determined by Coomassie Brilliant Blue staining. Open circles, polyribosomes from brain; solid circles, polyribosomes from liver.

The concentration of S-100 protein in whole brain of the rat reaches adult levels when the rat is 25 days old (*8*). Using complement fixation, we have found that, in adult rats, about 0.5 to 0.8 percent of the soluble protein of brain is S-100 protein. A relatively low rate of S-100 protein synthesis is suggested by this amount of protein present in rat brain, and by the low degradation rate of S-100 protein recently reported (*3, 4*). After we submitted this manuscript, a report by Zomzely-Neurath *et al.* was published (*10*) in which they reached substantially the same conclusions we have described. Zomzely-Neurath *et al.*, using a method of analysis identical to ours, found about 0.3 percent of the radioactivity released from polyribosomes of the rat brain, and precipitable by trichloroacetic acid, was present in S-100 protein. Unlike previous reports, which proposed that a large percentage of protein synthesized by brain polyribosomes is S-100 protein (*5*), our data and that of Zomzely-Neurath *et al.* indicate that S-100 protein synthesis comprises only a small percentage of the total released protein synthesized on rat brain polyribosomes.

MICHAEL P. LERNER
HARVEY R. HERSCHMAN
Department of Biological Chemistry and Laboratory of Nuclear Medicine and Radiation Biology, School of Medicine, University of California, Los Angeles 90024

References and Notes

1. B. W. Moore, *Biochem. Biophys. Res. Commun.* **19**, 739 (1965).
2. B. S. McEwen and H. Hyden, *J. Neurochem.* **13**, 823 (1966).
3. T. J. Cicero and B. W. Moore, *Science* **169**, 1333 (1970).
4. H. R. Herschman, *J. Biol. Chem.* **246**, 7569 (1971).
5. A. L. Rubin and K. H. Stenzel, *Proc. Nat. Acad. Sci. U.S.A.* **53**, 963 (1965).
6. The specific activities of the tritiated amino acids (Schwarz) were: [4,5-^{3}H]leucine, 51 c/mmole; [2,3-^{3}H]lysine, 42 c/mmole; [2,3-^{3}H]-valine, 17 c/mmole; [^{3}H]phenylalanine, 6.5 c/mmole.
7. Phosphate-buffered saline consists of 0.15*M* NaCl and 0.01*M* PO_4, *p*H 7.0.
8. H. R. Herschman, L. Levine, J. de Vellis, *J. Neurochem.* **18**, 629 (1971).
9. R. Mans and G. D. Novelli, *Arch. Biochem. Biophys.* **94**, 48 (1961).
10. C. Zomzely-Neurath, C. York, B. W. Moore, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 2326 (1972).
11. Supported by NSF grant GB-30518 and in part by contract AT (04-1) GEN 12 between the U.S. Atomic Energy Commission and the University of California. M.P.L. was supported in part by NIH postdoctoral fellowship 1 FO2 NS 45571-02.

31 July 1972