Table 1. Base composition of chick embryo RNA and DNA. C, cytosine; A, adenine; G, guanine; U, uracil; and T, thymine.

Mol. size (S)	Age of embryo (days)	Base (% of total base) C	A	G	U(T)	G+C
		RNA				
28	7	28	19	34	18	62
	3	24	18	36	22	60
16	7	25	24	29	22	54
	3	25	24	30	21	55
40	7	25	23	28	25	53
		DNA				
	7	22	28	22	28	44

served, and the ratio of optical densities at 260 to 280 mμ indicated contamination with non-nucleic acid material in this region of the gradient.

Figure 1 shows that relatively long periods of exposure to isotope (P^{32} for 4 to 18 hours) result in incorporation of radioactivity into ribosomal RNA of similar size in embryos of from 4 hours to 7 days of age. This represents a striking difference in embryogenesis between the chick embryo and the frog embryo. Brown and Caston (*9*) have recently shown that only one type of ribosomal RNA is formed during the first 96 hours of development in the frog egg. Presumably one of the factors underlying this difference is that during early differentiation in the chick embryo the cell mass is increasing, while in the frog egg, individual cells are being formed within an already existing mass.

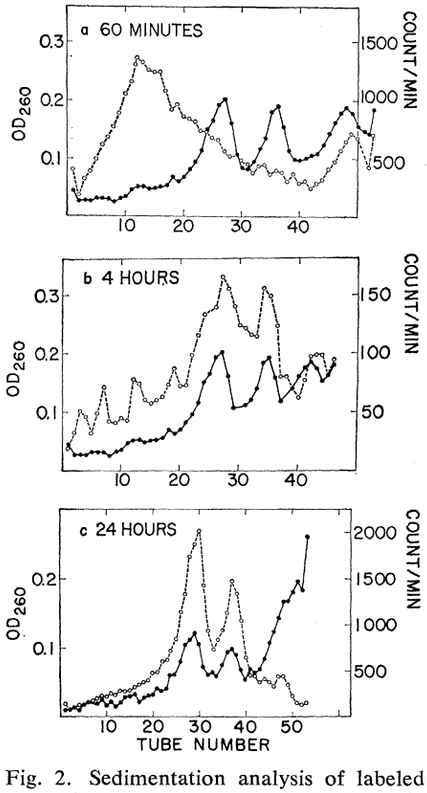

Fig. 2. Sedimentation analysis of labeled RNA from a suspension of 7-day chick-embryo cells. A suspension of cells from three 7-day embryos prepared as described were incubated with 6 μc C^{14}-uridine (33 μc/μmole) in 30 ml Eagle's medium in (*a*) 1 hour, (*b*) 4 hours, and (*c*) 24 hours. RNA was then extracted and analyzed on sucrose gradients (*5*). Solid lines ·—·, optical density; broken lines o---o, count/min.

In addition to similarity in size from day 1 to 7, ribosomal RNA was similar in composition when 3- and 7-day chick material was studied (Table 1). The 28*S* RNA from material obtained at both 3 and 7 days had identical base ratios, as did also the 16*S* RNA. There was a distinct difference, however, between the base composition of 28*S* and 16*S* molecules at both times. This agrees with the recent finding (*10*) that the two ribosomal RNA molecules in *Escherichia coli* are derived from separate loci on the DNA template.

When animal cells in culture are exposed to P^{32} orthophosphate or uridine-2-C^{14}, radioactivity accumulates in a precursor of high molecular weight (35*S* and 45*S*) before it appears in ribosomal RNA. Actinomycin D, which stops further RNA synthesis (*11*), has been used to follow the change of precursors from 45*S* and 35*S* to 28*S* and 16*S* RNA in the absence of further RNA synthesis (*5*).

With labeling periods of 30 to 60 minutes, material of large molecular weight (40*S*) is preferentially labeled also in the chick embryo (Fig. 2). The guanine-cytosine (G-C) content of the 40*S* material (Table 1) was similar to that of 16*S* ribosomal RNA and intermediate between that of 28*S* ribosomal RNA and DNA. Attempts to use actinomycin D to follow the fate of the 40*S* RNA in chick-embryo cells in suspension, as was done with HeLa cells, have been unsuccessful owing to the disintegration of the cells within 4 hours in medium containing actinomycin. Thus, although it is quite likely that some of the 40*S* material is ribosomal precursor RNA, the fraction remains uncertain.

The results of these experiments indicate a similarity of ribosomal RNA throughout embryogenesis. Although subtle changes in ribosomes during development could not be detected by our techniques, it seems reasonable to search for different messenger RNA molecules as the basis for the formation of different proteins which appear as development proceeds (*12*).

A. Martin Lerner*
Eugene Bell
James E. Darnell, Jr.
Department of Biology, Massachusetts Institute of Technology, Cambridge 39

References and Notes

1. *Cold Spring Harbor Symp. Quant. Biol.*, 26 (1961).
2. J. R. Warner, P. Knopf, A. Rich, *Proc. Natl. Acad. Sci. U.S.* **49**, 122 (1963).
3. S. Penman, K. Scherrer, Y. Becker, J. E. Darnell, *ibid.* **49**, 654 (1963).
4. H. Eagle, *Science* **130**, 432 (1959).
5. K. Scherrer and J. E. Darnell, *Biochem. Biophys. Research Commun.* **7**, 486 (1962). K. Scherrer, H. Latham, J. E. Darnell, *Proc. Natl. Acad. Sci. U.S.* **49**, 240 (1963).
6. J. Marmur, *J. Mol. Biol.* **3**, 208 (1961).
7. A. Marshak and H. J. Vogel, *J. Biol. Chem.* **189**, 597 (1951).
8. R. J. Britten and R. B. Roberts, *Science* **131**, 32 (1960).
9. D. D. Brown and J. D. Caston, *Develop. Biol.* **5**, 412 (1962).
10. S. A. Yankofsky and S. Spiegelman, *Proc. Natl. Acad. Sci. U.S.* **49**, 538 (1963).
11. E. Reich, R. M. Franklin, A. J. Shatkin, E. F. Tatum, *ibid.* **48**, (1962).
12. Supported by the U.S. Public Health Service and the National Science Foundation. We thank Mrs. M. Heeter and Mrs. P. Goodwin for technical assistance.
* Fellow of the Medical Foundation, Inc. Present address: Department of Medicine and Microbiology, Wayne State University College of Medicine, Detroit 7, Mich.

15 July 1963

Tremorine: Its Effect on Amines of the Central Nervous System

Abstract. *The administration of tremogenic doses of Tremorine, 1,4-dipyrrolidino-2-butyne, is followed by a significant decrease in the concentration of norepinephrine in the brain stem of three common laboratory species. The change in the concentration proceeds at a rate which coincides with the occurrence of the tremor in each of these species. In the rat, the change in norepinephrine is followed by a progressive increase in the concentration of 5-hydroxytryptamine in the brain stem. Bilateral adrenalectomy in the rat enhances the Tremorine-induced changes in the concentration of norepinephrine and antagonizes the increase in the concentration of 5-hydroxytryptamine.*

When Tremorine is administered to laboratory animals, it causes a condition which simulates many aspects of Parkinsonism, in that it produces tremor, rigidity, and a predominance of parasympathomimetic signs (*1*). It has been shown that this compound affects

both divisions of the autonomic nervous system and that some of the effects obtained may be attributed to ganglionic stimulation (*2*). Recent clinical studies have indicated that the metabolism of certain amines in the central nervous system are altered in patients with Parkinson's disease (*3, 4, 5*). Postmortem examination of the basal ganglia of such individuals reveals that the concentration of norepinephrine, dopamine, and 5-hydroxytryptamine is decreased (*3, 4*). More recently it has been shown that the administration of *l*-DOPA provides transient relief from some of the motor defects of Parkinsonism (*6*). In view of the acute Parkinson-like state produced with Tremorine in laboratory animals, is was of interest to determine whether this compound affected the amines of the central nervous system.

The concentrations of catecholamines and of 5-hydroxytryptamine in the brains of the animals were determined by the trihydroxyindole fluorimetric method of Shore and Olin (*7*) and the method of Bogdanski (*8*), respectively, or by using the single extraction method of Mead and Finger (*9*). Tremorine was administered intraperitoneally, 15 to 20 mg/kg of body weight; controls either received saline or were untreated. Animals were decapitated by guillotine 5 to 90 minutes after treatment. Brains were removed, dissected, and weighed, and were then placed in cold HCl, homogenized, and assayed.

The variability in concentration of brain catecholamines increased considerably when animals unadapted to a laboratory environment were used. Motor and neurochemical changes were generally found to be more pronounced in older than in younger animals. Data obtained for norepinephrine are summarized in Table 1. In the rat, mouse, and guinea pig, there was a significant decrease in the concentration of norepinephrine in the brain stem after the administration of Tremorine. In the rat, this decrease was maximal (−24.8 percent) in 60 minutes, and in the mouse, it was maximal (−16.4 percent) in 5 minutes. In the guinea pig, the decrease in the concentration was 24.7 percent after 15 minutes and 21.9 percent after 30 minutes; the difference between these two values is not significant. Similar changes occurred in the cerebral cortex of the rat and in the cerebral and cerebellar cortex of the guinea pig. The rise in the concentration of norepinephrine in the cerebral cortex of the mouse is not significant. After 90 minutes in the rat, and 15 minutes in the mouse, the concentrations in the brain stem were still significantly decreased. The rate at which these changes proceeded correlates well with the time course of Tremorine tremor. Tremor activity in the rat (*10*), for example, begins within 15 minutes after intraperitoneal administration of Tremorine, rises to a maximum within an hour and then disappears slowly over a period of several hours.

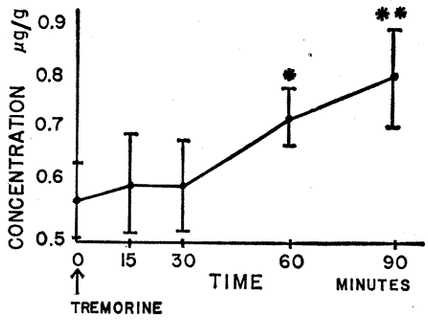

Fig. 1. The effect of Tremorine on the concentration of 5-hydroxytryptamine in the brain stem of the rat, expressed as μg/g of fresh tissue. Each point is the mean for 5 animals; the vertical bars represent the standard deviation for each group. * Significant increase, $p < .05$; ** highly significant increase, $p < .01$.

The concentration of epinephrine in the brain stem of the rat also decreased (by as much as 18.1 percent), 30 and 60 minutes after administration of Tremorine, but the data were not statistically significant because of the variability in the small amounts of the amine found in this region.

In a fourth species, the rabbit, in which Tremorine does not produce tremor, the concentration of norepinephrine did not change significantly in the brain stem, in fact the mean tissue levels in the experimental group were indistinguishable from those in the control group. A decrease of borderline significance did occur in the cerebellum (percentage change: 13 percent, $p < .1$, $> .05$).

In the adrenalectomized rat the change in the concentration of norepinephrine in the brain stem was greater than in unoperated animals, for example, in one of the groups (12 animals: 6 experimental, 6 control), 1 week after bilateral adrenalectomy, 20 mg/kg of Tremorine decreased the concentration of catecholamines by 31.4 percent in 90 minutes (compared with 15.5 percent in unoperated animals). However, the tremor of these adrenalectomized rats was less pronounced.

Figure 1 depicts the change in the concentration of 5-hydroxytryptamine in the brain stem of the rat after intraperitoneal administration of 20 mg of Tremorine per kilogram of body weight. A significant rise was evident after 60 minutes, and after 90 minutes the concentration was 40.8 percent above that in the control. After bilateral adrenalectomy, the concentration

Table 1. Effect of Tremorine on the concentration of norepinephrine in the central nervous system, expressed as micrograms of norepinephrine per gram of wet tissue. Each determination was made on a single animal, except in the mouse, where each determination was made on a pooled pair of brain stems or cerebral cortices. Figures in parentheses indicate number of determinations.

Tissue	Time after treatment (minutes)	Norepinephrine (μg/g)		
		Control (mean ± standard error)	After Tremorine (mean ± standard error)	*p* value
		Rat		
Brain stem	15	0.877 ± 0.031 (6)	0.763 ± 0.046 (4)	>.05
Brain stem	30	1.140 ± .116 (4)	1.040 ± .116 (4)	>.05
Brain stem	45	0.918 ± .024 (12)	0.721 ± .031 (12)	<.001
Brain stem	60	1.084 ± .024 (24)	0.815 ± .035 (24)	<.001
Cortex	60	0.623 ± .038 (5)	0.500 ± .041 (5)	<.05
Brain stem	90	0.990 ± .014 (9)	0.837 ± .028 (9)	<.01
		Mouse		
Brain stem	5	1.282 ± 0.026 (12)	1.072 ± 0.024 (12)	<.05
Cortex	5	0.462 ± .040 (6)	0.532 ± .022 (6)	>.05
Brain stem	15	1.260 ± .040 (6)	1.080 ± .038 (6)	<.01
Brain stem	30	1.124 ± .032 (6)	1.045 ± .039 (5)	>.05
		Guinea pig		
Brain stem	15	0.646 ± 0.047 (5)	0.487 ± 0.024 (6)	<.01
Cerebellum	15	0.313 ± .017 (6)	0.263 ± .011 (6)	<.01
Brain stem	30	0.630 ± .039 (6)	0.492 ± .026 (7)	<.01
Cortex	30	0.247 ± .048 (6)	0.189 ± .050 (7)	>.05

of 5-hydroxytryptamine was not significantly changed by the administration of Tremorine.

Oxotremorine, a metabolite of Tremorine (*11*) was also investigated. When 200 μg/kg was injected intraperitoneally, the concentration of norepinephrine in the brain stem of the rat decreased, while the concentration of 5-hydroxytryptamine increased after 60 minutes, but the limited quantity we were able to obtain (*12*) did not allow further investigation.

These results are in partial agreement with recent clinical findings which suggest that there might be some biochemical malfunction in Parkinson's disease, especially with regard to the metabolism of catecholamines. They also appear to be relevant to findings in drug-induced extrapyramidal reactions (*13*). Our results with 5-hydroxytryptamine are not in agreement with those of Bernheimer, Birkmayer, and Hornykiewicz (*4*) who used brains from patients with Parkinson's disease, but appear to find support in the studies of Domer and Feldberg (*14*) who showed that intraventricular administration of 5-hydroxytryptamine enhanced, while catecholamines depressed, drug-induced tremors in the cat.

When Tremorine is administered to the adrenalectomized rat, the depletion of norepinephrine is increased, the concentration of 5-hydroxytryptamine is unaltered, and tremor is diminished; this suggests that Tremorine tremor, and perhaps parkinsonian tremor, involves both central and peripheral mechanisms, and that those in the periphery modulate those in the central nervous system by feedback.

The studies mentioned above also suggest why present-day pharmacotherapy of Parkinsonism is less than ideal. It is apparent that the basic disorder has not been clearly determined and that current attempts at correction offer only partial solutions of the problem. The exact role in motor function of the biogenic amines studied is still to be elucidated. It is significant that Tremorine, which gives rise to a condition in laboratory animals that simulates the Parkinson syndrome so remarkably, also reproduces acutely some of the biochemical features of this disease (see *15*).

ALEXANDER H. FRIEDMAN
ROBERT J. AYLESWORTH
GERTRUD FRIEDMAN
Department of Pharmacology and Toxicology, University of Wisconsin Medical School, Madison 6

References and Notes

1. G. M. Everett, L. E. Blockus, I. M. Shepperd, *Science* **124**, 79 (1956).
2. A. H. Friedman and C. M. Smith, *J. Pharmacol. Exptl. Therap.* **135**, 62 (1962).
3. H. Ehringer and O. Hornykiewicz, *Klin. Wochschr.* **38**, 1236 (1960).
4. H. Bernheimer, W. Birkmayer, O. Hornykiewicz, **39**, 1056 (1961).
5. A. Barbeau, G. F. Murphy, T. L. Sourkes, *Science* **133**, 1706 (1961).
6. W. Birkmayer and O. Hornykiewicz, *Wien. Klin. Wochschr.* **73**, 787 (1961).
7. P. Shore and J. S. Olin, *J. Pharmacol. Exptl. Therap.* **122**, 295 (1958).
8. D. F. Bogdanski, A. Pletscher, B. B. Brodie, S. Udenfriend, *ibid.* **117**, 82 (1956).
9. J. A. R. Mead and K. F. Finger, *Biochem. Pharmacol.* **6**, 52 (1961).
10. Tremor activity was assessed by an arbitrary scale which evaluates the degree of tremor as well as the anatomical region involved. For example, mild tremor restricted to the ears is rated at 1+.
11. J. J. Kocis and R. M. Welch, *Pharmacologist* **2**, 87 (1960).
12. We gratefully acknowledge the gift of oxotremorine (as the picrolonate) from W. L. Haslett, University of California Medical Center, Los Angeles.
13. P. L. McGeer, *et al.*, *J. Am. Med. Assoc.* **177**, 101 (1956).
14. F. R. Domer and W. Feldberg, *Brit. J. Pharmacol.* **15**, 578 (1960).
15. A preliminary report of this study has appeared: A. H. Friedman, *Federation Proc.* **22**, 272 (1963). This study was supported by U.S. Public Health Service grant NB 03139-03.

12 July 1963

Antigenic Histamine Release from Passively Sensitized Human Leukocytes

Abstract. *With histamine release as an indicator, consistent passive sensitization of normal human leukocytes to specific antigen could be achieved with human serum, fresh or stored at −70°C, from allergic individuals. With certain sera, dilution slowed the rate of sensitization but increased eventual histamine release.*

When specific antigen is incubated with blood from a person with atopic allergy, histamine is released from the leukocytes (primarily basophiles) into the plasma (*1*). Since the same reaction occurs if antigen is added to washed leukocytes, free skin-sensitizing antibody has no apparent role in the reaction; apparently, the leukocytes have been sensitized optimally in vivo (*2*). Accordingly, the immunologic behavior of skin-sensitizing antibody in vitro can be studied only by passive sensitization of normal cells. However, the results of earlier attempts to accomplish such passive sensitization have been disappointing (*3*). In the one published report in which histamine release was the indicator, Middleton was successful in only three of ten experiments (*4*).

In our study, certain modifications have made possible the consistent and reproducible passible sensitization of normal leukocytes. Serum was donated by twelve persons who were known to be allergic to Timothy pollen by the usual criteria as well as by release in vitro of histamine with their own leukocytes. The serum was stored at −70°C until used. Four nonallergic persons served as cell donors. For each experiment, nonallergic blood, collected in a siliconized syringe containing heparin, was centrifuged immediately. The buffy coat was removed, washed twice with Tyrode's solution, and separate samples were suspended in 2-ml volumes of Tyrode's solution, normal serum, or allergic serum. The suspensions were incubated in a water bath at 37°C for periods from 30 minutes to 6 hours. Timothy-pollen antigen was then added to make a concentration of 0.03 μg of protein nitrogen per milliliter; incubation was continued for one hour. After centrifugation, each cell-free supernatant solution was assayed for histamine content by a method combining two modifications of the Shore fluorometric procedure (*5*).

In each of 40 experiments, cells incubated with allergic serum released significantly more histamine after addition of antigen than those incubated with normal serum or with Tyrode's solution. These experiments included 16 different donor-donor combinations (Table 1). The effect of brief heating is shown in Table 2. All allergic sera were inactivated by heating at 56°C for 45 minutes, but their specific activity, as shown by passive transfer to skin, was not appreciably diminished. Similarly, freezing at ordinary temperatures (−10° to −18°C) did not preserve activity; consistent results were obtained only with fresh sera or with sera stored at −70°C. Apparently, complement was not necessary for sensitization, since the chelating agent, EDTA (.01*M*), did not block sensitization by active serum and fresh normal

Table 1. Histamine released from normal cell suspensions after incubating 4 hours with diluted (50 percent) serum and 1 hour with Timothy antigen (.03 μg protein nitrogen per milliliter). The total cellular histamine was 85 μg/liter.

Cell samples	Incubation		Histamine (μg/liter)
	4 hours	1 hour	
1–2	Tyrode's	Antigen	5.9
3–4	A.S.*	Antigen	39.5
5–6	A.S.	No antigen	4.6
7–8	N.S.†	Antigen	5.1

* Allergic serum (pt. *Sh.*). † Normal serum.