adenosine-3′,5′-monophosphate (cyclic AMP) which is a naturally occurring intracellular constituent (*7*). Indeed, cyclic AMP appears to mediate the effects of several hormones including the action of catecholamines and glucagon on liver, adrenocorticotropin on the adrenals, and vasopressin on toad bladder (*8*). In *Calliphora,* the effect of 5-HT on salivary glands may also be mediated by cyclic AMP because this substance can stimulate secretion (Fig. 1), whereas related compounds such as adenosine, adenylic acid, and adenosine triphosphate are ineffective over a similar range of concentration. The dose-response curve resembles that of 5-HT, except that the concentration of cyclic AMP needed is much greater. Where the intracellular levels of cyclic AMP have been measured, the concentration ranges between 10^{-8} and $10^{-6}M$ (*9*). Perhaps in salivary glands the cell membrane acts as a diffusion barrier to cyclic AMP and this accounts for the high concentration needed to stimulate secretion.

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Intrastrain Differences in Serotonin and Norepinephrine in Discrete Areas of Rat Brain

Abstract. *Determination of levels of serotonin and norepinephrine in various brain areas of male Sprague-Dawley rats obtained from four different breeder-suppliers showed considerably different basal levels among the various groups, as well as differences in response to monoamine oxidase inhibitors.*

A considerable body of literature has evolved since the first reported measurements of serotonin (5-HT) (*1*) and norepinephrine (NE) (*2*) in mammalian brain. In general, most reports describe levels of the amines in whole brain or brain stem; very few reports have appeared on levels of 5-HT or NE in discrete brain areas. The picture is further complicated by reports confirming species and strain differences in brain amine levels (*3*), and by sensitivity limitations of methodology (*4*). We have examined the basal levels of 5-HT and NE in several discrete brain areas of Sprague-Dawley rats, using a more sensitive assay procedure developed in this laboratory (*4*). The results indicate considerable differences in levels of 5-HT and NE in brain areas of animals of the same strain obtained from different suppliers.

Adult, male Sprague-Dawley rats (60 to 80 days old, 300 to 350 g in body weight) were obtained from four commercial sources: Harlan Laboratories, Indianapolis, Hormone Assay Laboratories, Chicago, Simonsen Laboratories, St. Paul, and Windsor Biology Gardens, Bloomington. The animals were maintained with free access to Purina Lab Chow and water for at least 7 days prior to experimental use. After decapitation of the rats, brains were removed, areas dissected, and 5-HT and NE were determined as previously described (*4*).

The normal levels of 5-HT and NE in brain areas of rats obtained from the various sources are presented in Table 1. The differences are striking. Levels of 5-HT and NE are remarkably consistent in the cerebellum of the various groups. However, in other areas, considerable differences are seen in normal amine levels. Values for whole brain reflect largely the differences in the cerebral hemispheres, the largest portion of the whole brain weight. Further examination of rats obtained from two of these sources, Hormone Assay Laboratories and Simonsen Laboratories, indicate that significant ($P < .05$) differences exist for NE in cerebral hemispheres, hypothalamus-thalamus, and midbrain, and for 5-HT in midbrain and medulla.

Additional studies of the elevation in brain 5-HT and NE levels induced by administration of the monoamine oxidase inhibitor, pargyline (40 mg/kg, intraperitoneally), to Hormone Assay and Simonsen animals are also shown. Under these conditions, the effect on brain amines at 30 minutes after the

Table 1. Concentrations (μg/g) of serotonin and norepinephrine in areas of rat brain. Values are means ± standard deviations. Abbreviations: *N*, number; CH, cerebral hemisphere; HTH, hypothalamus; WB, whole brain.

Supplier	*N*	Brain area					
		CH	Cerebellum	HTH	Midbrain	Medulla	WB
		Serotonin					
Harlan	8	0.77 ± .04	0.24 ± .01	1.49 ± .44	1.05 ± .10	0.91 ± .07	0.76 ± .09
Hormone Assay	7	0.86 ± .05	0.24 ± .02	1.40 ± .10	1.35 ± .09	1.23 ± .10	0.88 ± .07
Simonsen	8	0.79 ± .07	0.22 ± .01	1.41 ± .10	1.16 ± .13	0.98 ± .13	0.79 ± .07
Windsor	4	0.89 ± .04	0.27 ± .02	1.48 ± .45	1.30 ± .05	1.23 ± .09	0.89 ± .05
Hormone Assay + pargyline	3	1.13 ± .09	0.29 ± .02	2.20 ± .10	1.77 ± .14	1.39 ± .13	1.14 ± .06
Simonsen + pargyline	3	1.08 ± .11	0.26 ± .04	1.91 ± .09	1.60 ± .09	1.26 ± .10	1.07 ± .09
		Norepineprine					
Harlan	8	0.40 ± .03	0.19 ± .02	2.37 ± .51	0.67 ± .05	0.70 ± .13	0.48 ± .05
Hormone Assay	7	0.57 ± .04	0.22 ± .04	3.12 ± .44	0.95 ± .07	1.07 ± .10	0.67 ± .05
Simonsen	8	0.43 ± .07	0.20 ± .03	2.01 ± .28	0.80 ± .09	0.93 ± .17	0.54 ± .07
Windsor	4	0.51 ± .07	0.21 ± .02	2.90 ± .44	0.83 ± .06	0.94 ± .06	0.60 ± .07
Hormone Assay + pargyline	3	0.68 ± .01	0.29 ± .04	3.34 ± .61	1.13 ± .07	1.14 ± .04	0.79 ± .03
Simonsen + pargyline	3	0.49 ± .04	0.29 ± .16	2.15 ± .51	0.91 ± .13	0.92 ± .12	0.59 ± .08

drug was also variable. For example, the whole brain values for 5-HT increase by 30 percent for Hormone Assay rats and 35 percent for Simonsen rats. However, the brain area changes range from an increase of + 13 percent for medulla of the Hormone Assay rats to + 57 percent for the hypothalamus of these rats. In the case of NE, the increases in whole brain levels are + 18 percent for the Hormone Assay rats and + 9 percent for the Simonsen rats. In contrast, the changes in NE in brain areas range from no change (actually a 1 percent decrease) in medulla of the Simonsen rats to an increase of + 45 percent in the cerebellum of Simonsen rats. The whole brain values most closely compare to the effects in cerebral hemispheres, the largest portion (by weight) of the whole brain.

These results contain implications of considerable significance to workers examining interactions of drugs with brain biogenic amines, and may serve to explain some of the apparent discrepancies that exist in the literature. As previously mentioned (*3*), differing amine levels have been reported in various species and strains of animals. However, no attention has been paid to possible differences within the same strain. For example, these results would seriously question the validity of extrapolating data obtained on biochemical effects of drugs influencing brain 5-HT and NE from one supply of rats to another, even though both are from the same strain. Similar questions might well be raised regarding attempts to relate biochemical data on the amines obtained in one laboratory with drug effects on animal behavior obtained in another.

The precise reason for the differences in basal levels is as yet unknown and will require further study. However, the similarity in response to pargyline suggests similarity in rates of biosynthesis and degradation of the amines. Thus, the marked differences in basal levels may reflect differences in storage sites.

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Prophage S2 Mutants in Haemophilus influenzae: A Technique for Their Production and Isolation

Abstract. *A procedure utilizing nitrosoguanidine has been developed to produce defective and temperature-sensitive mutants of prophage (S2) in lysogenic* Haemophilus influenzae. *The system should be generally applicable to all temperate phage systems. At saturating concentrations of phage DNA, more than 25 percent of recipient mutant lysogenic bacteria can be transformed to the wild type.*

Mutants of virulent bacteriophage are ordinarily obtained by treating free phage (*1*) or infected cells (*2*) with mutagen. The mutants so obtained fall mainly into three categories—(i) plaque-morphology mutants, (ii) mutants having distinct physical characteristics (such as an altered bouyant density), and (iii) conditional-lethal mutants (including temperature-sensitive and host-range mutants). Totally lethal mutations cannot be obtained (except perhaps transiently) by mutations originating in the phage unless the lethal mutant is not dominant, and unless it can be separated from a complementary phage after mixed infection (*3*). However, it is possible to propagate all types of mutations, including lethals, in temperate bacteriophage if the mutations are introduced into the prophage, providing only that the ability to be maintained as a prophage is not lost. This report describes a technique by which lysogenic cells of *Haemophilus influenzae* are mutagenized with nitrosoguanidine and the mutant S2 prophage is selected by replica plating of the lysogenic cells onto plates overlaid with indicator bacteria (lawn plates). Additional advantages of this technique are the potent mutagenicity of nitrosoguanidine in cells (compared with its feeble effects on free phage); quick and easy scanning for mutants, yielding many kinds of mutants in large numbers; and the general applicability of the technique to all temperate phage systems, including those for which no host suppressor mutants are yet known.

A stock of mutated lysogenic cells was prepared according to the following procedure [which is an adaptation of the procedures of Galinski and Goodgal, and Michalka and Goodgal (*4*)].

1) Wild-type lysogenic cells were grown to about 10^9 per milliliter (measured by turbidity) at 37°C in 75 ml of sBHI [brain-heart infusion broth (Difco) supplemented with 10 μg of hemin and 2 μg of nicotinamide adenine dinucleotide (NAD) per milliliter].

2) The cells were washed once in saline, and resuspended in 1/20th volume (3.75 ml) of saline-acetate buffer (which consists of 1 volume of 0.1*M* sodium acetate, *p*H 5.0, added to 5 volumes of saline).

3) A solution of nitrosoguanidine (0.75 ml) [2 mg of *N*-methyl-*N*-nitroso-*N'*-nitro-guanidine (*5*) per milliliter in saline-acetate buffer] was added to 3.75 ml of a cell suspension in saline-acetate buffer. The final *p*H of this suspension is about 6.0.

4) Suspended cells were incubated with mutagen for 10 minutes at 37°C, and the reaction was terminated by the addition of an equal volume of ice-cold BHI broth (no hemin or NAD). The cells were centrifuged and washed with more cold BHI broth.

5) Following resuspension in 100 ml of fresh sBHI broth, cells were incubated with shaking at 31°C for 2½ hours (four to five generations).

6) The cells were centrifuged, resuspended in BHI broth plus 15 percent glycerol, and stored at − 70°C in several small portions.

7) Working stocks of mutated cells were prepared as needed from the above by thawing a portion, diluting into fresh sBHI broth, and growing for four or five generations at 31°C. These cells were resuspended in fresh BHI broth plus 15 percent glycerol and stored in small portions at −70°C.

Mutant prophages were identified by replica plating from "working stock" as follows: (i) Plates of sBHI agar were spread with diluted working stock of mutated lysogenic cells so that each plate received about 150 to 300 viable cells. (ii) The plates were incubated at 31°C for 24 hours, or until colonies reached about 1 to 2 mm in diameter.