in the number of positive cultures (Table 1). When these data were analyzed with regard to sample type, however, differences were seen—prostate and vas deferens samples had a higher percentage of positives than did urethral swabs (Table 2). All control swabs were negative. It should be noted that the higher isolation rates found in this study, as compared with rates given in a previous report (*7*), could have been caused by dissimilar methods of collecting samples.

It is interesting that herpesvirus was isolated from patients with diagnosed cancer. Of the 20 cancer patients (ages 45 to 70), four positive cultures were obtained from urethral swabs, which is a much higher incidence than in the total positive cultures found in urethral swab specimens.

Although well-controlled epidemiologic studies are required, this investigation indicates that the male genitourinary tract, unlike the female genital tract, serves as a reservoir for herpesvirus. The relatively high incidence of positive cultures found in specimens obtained from deeper tissue, such as prostate gland and vas deferens, is consistent with reports of herpesvirus in glandular tissue, such as the lacrimal gland of patients with recurrent herpes keratitis (*8*).

Since herpesvirus can persist in the male genitourinary tract in the absence of overt disease, it provides a reservoir for the venereal transmission of the virus and indicates that the relationship of this virus to prostatic cancer or other male genitourinary disease deserves further study.

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Adaptation of the Adrenal Medulla: Sustained Increase in Choline Acetyltransferase by Psychosocial Stimulation

Abstract. *Sustained increases were produced in adrenal choline acetyltransferase of individually caged mice by placing them into groups for 10 or 15 minutes daily for 7 to 10 days. They were left undisturbed in their individual cages for the remainder of each day. As in previous experiments of similar design, adrenal catecholamines and adrenal weight were also increased, although body weight was not affected.*

Emotional arousal enhances the release of catecholamines from the adrenal medulla (*1*). Even short daily periods of intense neural stimulation cause sustained adaptive changes in the chromaffin tissue of the adrenal medulla (*2, 3*). Activities of adrenal enzymes for catecholamine biosynthesis are increased by neural stimulation only if the preganglionic innervation of the adrenal gland is intact (*4*). However, the activity of tyrosine hydroxylase, the rate-limiting enzyme for the biosynthesis of the catecholamines, is increased even in denervated adrenal glands by repeated injections of acetylcholine (*5*). Further, methedrine, injected into rats for two or more consecutive days, stimulates the release of adrenal catecholamines, and increases the activity of choline acetyltransferase as well as that of tyrosine hydroxylase (*6*). Fighting depletes catecholamines from the adrenal medulla (*7*), but repeated fighting causes adaptive increases in adrenal catecholamines (*2*) and in the enzymes for their biosynthesis (*8*). From these observations, we reasoned that brief periods of daily fighting should increase the activity of choline acetyltransferase in the adrenal glands of mice.

Male mice were received from the supplier at 4 weeks of age and were divided into two groups. Mice for experiment 1 were immediately caged individually for 4 weeks to make them aggressive (*9*). During the last 10 days of this period, three to eight test animals were placed together for 10 to 15 minutes each afternoon to permit them to fight. They were killed by decapitation 18 hours after the last session. Mice for experiment 2 were kept in groups of six until 3 months of age, at which time they were individually housed for 1 month. Test animals were placed in groups for 10 to 15 minutes on each of the last 7 days of this period and were killed by decapitation 18 hours after the last session. Both adrenals from each animal were assayed for choline acetyltransferase and cholinesterase or for epinephrine and norepinephrine. Whole brains (experiment 1) were assayed for choline acetyltransferase and cholinesterase (*10*).

Mice caged individually for 1 month immediately after weaning (experiment 1) fought intensely when initially placed in groups. However, as in previous experiments (*2*), the intensity and frequency of fighting declined progressively with repeated exposures to the group situation; during the last three of the ten daily sessions they fought little or not at all. Mice that lived in groups for 2 months before being caged individually for 4 weeks (experiment 2) demonstrated increased motor and exploratory activity during the short periods of daily grouping; but even initially they made only weak, sporadic attempts to fight, and fighting was not observed at all during the last five of the seven daily periods of grouping.

Both total choline acetyltransferase activity of the paired adrenals and enzyme activity relative to protein were greater in the socially stimulated mice than in controls in both experiments (Table 1). The differences in control choline acetyltransferase activity in the two experiments were due to the differences in assay conditions (*10*). Adrenal cholinesterase activity was not changed by social stimulation (*11*). As in earlier experiments of similar design (*2*) adrenal catecholamines and adrenal weight were significantly greater in the stimulated mice than in controls, although body weight was not different (*12*). Neither choline acetyltransferase activity nor cholinesterase activity was greater in brains of stimulated mice as compared to controls (*13*). The latter results complement those of Consolo and Valzelli (*14*), who reported no difference

Table 1. Effects of intermittent psychosocial stimulation. Male mice, previously caged individually for 1 month, were subjected to the social stimulation of grouping for 10 to 15 minutes daily; control mice were not grouped. Results are given as the mean ± standard error of mean for 10 to 12 mice. Data were analyzed by Student's *t*-test (one-tailed, for unpaired data). Mice in each experiment were born within a 2-day period, and were 2 months of age when killed in experiment 1 and 4 months of age in experiment 2. Enzyme activity is expressed as quantity of acetylcholine formed.

Group	Choline acetyltransferase		Catecholamines (micrograms per left adrenal)		Adrenal weight (mg)
	Micromoles per gram of protein per hour	Nanomoles per adrenal pair per hour	Epinephrine	Norepinephrine	
			Experiment 1		
Control	10.2 ± 0.44	4.8 ± 0.51	3.2 ± 0.16	1.6 ± 0.19	3.4 ± 0.17
Experimental	11.4 ± 0.32	6.0 ± 0.30	5.6 ± 0.25	2.7 ± 0.19	4.0 ± 0.20
Difference	+12%	+25%	+75%	+69%	+18%
	($t = 2.15$; $P < .025$)	($t = 1.99$; $P < .05$)	($t = 8.15$; $P < .001$)	($t = 4.13$; $P < .001$)	($t = 2.36$; $P < .025$)
			Experiment 2		
Control	15.3 ± 1.03	8.4 ± 0.64	3.7 ± 0.20	1.6 ± 0.34	3.5 ± 0.12
Experimental	18.0 ± 0.88	12.5 ± 0.88	6.2 ± 0.35	3.1 ± 0.39	4.5 ± 0.22
Difference	+18%	+49%	+67%	+94%	+29%
	($t = 1.96$; $P < .05$)	($t = 3.71$; $P < .005$)	($t = 6.20$; $P < .001$)	($t = 2.92$ $P < .005$)	($t = 3.79$; $P < .005$)

in choline acetyltransferase activity in the brains of grouped and isolated mice, but our experiments differ from theirs in that our individually caged mice were intermittently grouped whereas theirs were not. However, others reported that social environment influences brain cholinesterase (*15*), and McKinney (*16*) suggested that adrenal catecholamines and environmental stimuli may have interacting influences upon brain cholinesterase.

We found intermittent grouping to cause similar increases in adrenal weight, adrenal catecholamines, and adrenal choline acetyltransferase whether the mice actually fought or not. This is consistent with other demonstrations that psychosocial stimulation may have important physiological effects—such as depletion of brainstem norepinephrine (*17*) and alterations of various endocrine secretions (*1*)—even though physical conflict is not involved.

The minimal amount of stimulation required to produce the observed changes, the rates at which changes develop or decay, and the durations of changes if no reinforcement is given are yet unknown. Likewise, the extent to which these changes may be due to handling of the mice and their transfer to a strange cage, as opposed to intermittent psychosocial stimulation per se, is not known.

We interpret our results to demonstrate that short daily periods of psychosocial stimulation may cause the preganglionic fibers of the adrenal medulla to undergo a sustained increase in capacity to synthesize acetylcholine. This adaptive change emphasizes the remarkable plasticity of the neuroendocrine system and the potency of the influence that emotional stimuli may exert upon it (*15, 18*).

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10. For choline acetyltransferase assay, 20 μl of buffer-substr → [R. E. McCaman and J. M. Hunt, *J. Neurochem.* **12**, 253 (1965)] containing either 80 μ*M* [1-^{14}C]acetyl coenzyme A (experiment 1) or 350 μ*M* [1-^{14}C]acetyl coenzyme A (experiment 2) were incubated with 2 μl of adrenal homogenate (20 μg of protein) prepared in distilled water. For cholinesterase assay, 20 μl of buffer-substrate (containing [1-^{14}C]acetylcholine iodide) was incubated with 2 μl of adrenal homogenate (1 to 2 mg of protein) [M. M. McCaman, L. R. Tomey, R. E. McCaman, *Life Sci.* **7**, 233 (1968)]. In each case, after the tubes were returned to the ice bath, 250 μl of 3-heptanone containing the sodium salt of tetraphenylboron (75 mg/ml) was added [F. Fonnum, *Biochem. J.* **115**, 465 (1969)]. The samples were mixed and centrifuged for 10 minutes at 900*g* at 2° to 4°C. A 200-μl portion of the 3-heptanone layer was placed in liquid scintillation vials for counting to estimate choline acetyltransferase; or the 3-heptanone layer was removed by aspiration, and 10 μl of the aqueous layer was placed in vials for counting to estimate cholinesterase. A 1-ml portion of Hyamine hydroxide (0.3*M* in methanol) and 15 ml of toluene (containing 5 g of diphenyloxazole and 0.1 g of 1,4-bis-methyl-5-phenyloxazol-2-yl benzene per liter) were added to each vial, and radioactivity was estimated in a Packard Tri-Carb liquid scintillation spectrometer. Methods have been published for measurement of protein [O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951)] and adrenal catecholamines (*2, 8*). The assay for brain enzymes was similar to that for the adrenal gland except that 5 to 6 μg of protein were used for choline acetyltransferase, and 0.4 to 0.5 μg of protein for cholinesterase.
11. Cholinesterase values (mean ± standard error of mean) were determined in paired adrenal glands in experiment 1. Specific activities (micromoles of acetylcholine hydrolyzed per gram of protein per hour) were 381 ± 37.1 (control) and 321 ± 32.0 (experimental); and total activities (micromoles of acetylcholine hydrolyzed per adrenal pair per hour) were 183 ± 22.9 (control) and 170 ± 14.9 (experimental). Cholinesterase was not assayed in experiment 2.
12. Body weights (mean ± S.E.M.) were 33.1 ± 0.43 g (control) and 33.3 ± 0.45 g (experimental) in experiment 1, and 38.0 ± 1.00 g (control) and 39.2 ± 0.58 g (experimental) in experiment 2.
13. In whole brains, cholinesterase values (micromoles of acetylcholine hydrolyzed per gram of protein per hour) were 6310 ± 834 for controls and 6423 ± 496 for experimental animals (five animals per group); and choline acetyltransferase activities (micromoles of acetylcholine formed per gram of protein per hour) were 58 ± 4.2 for controls and 67.7 ± 3.0 for experimental animals (12 animals per group).
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