3 weeks after first appearance of plasmodia in gills. Immediately prior to the kill 70 to 75 percent of living oysters have been found infected with *M. nelsoni*. Incidence in gapers (recently dead oysters) during the epizoötics is commonly 100 percent.

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Sleep Deprivation and Brain Acetylcholine

Abstract. *Rats deprived of D-state sleep (and, to some extent, of slow-wave sleep) for 96 hours show a significant fall in brain acetylcholine in the telencephalon; there were no significant changes in the diencephalon and brain stem. Restraint stress and activity wheel stress produced no significant change in acetylcholine levels in any of these regions; the telencephalic response to sleep deprivation, therefore, cannot be attributed to nonspecific stress. The effects of D-state deprivation and the psychoactive anticholinergic drugs on telencephalic acetylcholine levels are similar.*

The neurochemical consequences of prolonged sleep deprivation have not been identified. While the precise behavioral effects of sleep deprivation vary somewhat among species, some of the psychophysiological effects represent an ill-defined state of "activation." In man prolonged sleep deprivation results in signs of task-induced activation (*1*). Specific deprivation of those regularly recurrent periods of sleep referred to as REM, paradoxical sleep, activated sleep, or the D-state is followed by a "pressure" toward D-state (*2*). This is manifested by an apparently compensatory increase in activated or D-state sleep. These periods, associated with dreaming in man, are characterized in most mammals by signs of increased activity in various physiological measures [cortical electroencephalogram (EEG), heart rate, respiration, temperature, extraocular movements] and relaxation of certain groups of muscles of the head and neck (*3*). Since the amount of acetylcholine in the brain varies predictably along the crude dimensions of overall brain activity, from sleep or anesthesia (elevated acetylcholine) to seizures (lowered acetylcholine) (*4*), it appeared probable—given measures in the appropriate brain regions and adequate procedures for assuring D-state deprivation—that changes in acetylcholine could be observed. In a study in which rats were partially sleep-deprived for 48 hours on a slowly moving wheel in a water tank, there were no changes in amount of acetylcholine in whole brain (*5*); such procedures, however, do not reliably prevent D-state sleep, and estimation of acetylcholine in whole brain alone could mask significant regional changes.

To assure deprivation of the D-state component of sleep, male rats (200 to 275 g) were isolated for 96 hours on wooden blocks (5 cm square) in 5 cm of water (*6*); the animal does not get wet as long as it does not relax the muscles of the neck and head. It was first confirmed in 5 hours of early afternoon recording of a group of rats with implanted electrodes that these rats normally have about 15 periods of D-state sleep, each lasting 3 to 4 minutes, and 165 minutes of slow-wave sleep. The deprivation procedure did not permit any periods of D-state sleep (evident both by observation and EEG monitoring), although the animals were able to obtain numerous brief periods of slow-wave sleep. Recordings of two animals after 24 hours of D-state deprivation revealed an increased length and frequency of D-states. Accordingly, in the present studies the animals were deprived almost completely of D-state and perhaps to some extent of slow-wave sleep.

Experimental animals were removed every 4 hours for feeding and watering for a 15- to 20-minute period during which they were kept awake. At the end of the 96-hour deprivation period animals were taken immediately from the island and killed, and the brains were rapidly removed and dissected by a modified near-freezing technique (*7*) in which a mixture of acetone and dry ice was used in place of liquid nitrogen. Three regions of the brain were dissected: telencephalon (cortex, hippocampus, and caudate); diencephalon (thalamus, hypothalamus); and caudal brain stem (posterior to the colliculi and rostral to the obex and without the cerebellum). Control animals were of closely matched weights and ages. Acetylcholine was extracted with two portions of citrate buffer (*8*). Samples were frozen and assayed within 72 hours with LSD-25–stimulated ($10^{-7}M$) clam heart (*9*). Control values were in good agreement with those recently reported for comparable rat brain regions measured by the clam heart assay (*7*).

A total of 19 D-state deprived animals were studied in three separate experiments (Table 1). The telencephalon of the experimental animals showed a significantly lower mean acetylcholine level—a decrease of 35 percent—compared with controls. The difference between the mean acetylcholine values for control and sleep-deprived groups was significant ($P<.01$) for each experiment as well as for the pooled data. There was a slight but insignificant change in the same direction for the diencephalon and no change in the mean values for the caudal brain stem. To evaluate the specificity of this find-

Table 1. Amounts of acetylcholine in parts of rat brain, after a D-state deprivation procedure, restraint stress, and activity wheel stress. Results are expressed as percent of the mean control value (from five animals or more) with the number of experimental animals in parentheses. Absolute control values (micrograms of acetylcholine chloride per gram of tissue) for the D-state deprivation procedure were, for telencephalon, 2.87 ± 0.21 (*13*); for diencephalon, 2.91 ± 0.31 (*13*); brainstem 1.80 ± 0.17 (*8*).

Condition	Control acetylcholine (%)		
	Telencephalon	Diencephalon	Brain stem
D-state deprivation	65 (19)*	89 (19)	96 (13)
Restraint	110 (8)	97 (8)	103 (8)
Activity	91 (5)	89 (5)	97 (5)

* $P < .01$; all tests for significance calculated from the actual mean values of control and experimental groups.

ing, two other deprivation conditions were studied in conjunction with the control and sleep-deprived animals. One group was deprived of food for 48 hours and for the final 24 hours was deprived of water and placed in restraint jackets. Another group was placed in a continuously moving activity wheel for 24 hours. Neither procedure led to changes in regional acetylcholine values. While other deprivation situations could be tested, the results suggest that D-state deprivation cannot be considered a nonspecific response associated with an unlimited variety of stressors. Nor do situations of extreme stress uniformly involve the same neurochemical systems. The D-state deprivation procedure did not lead to significant changes in levels of norepinephrine and serotonin, whereas certain other stressors do (*10*). The specific components in various stress situations which underlie shifts in one or another neurochemical system in various brain regions have not been extensively explored.

Direct measures of neurochemical changes underlying the D-state per se are not available, although some control of the sleep-dream cycle has been linked to cholinergic processes (*3*, *11*) and to the buildup and metabolism of serotonin (*12*). Nor is the reason clear for localization in the telencephalon of drug or deprivation-induced changes in acetylcholine. Regional differences in choline acetyltransferase (choline acetylase) activity may indicate less efficient biochemical controls of acetylcholine in neural systems which are phylogenetically advanced (*13*).

When the D-state deprivation procedure was terminated, the rats appeared agitated and aggressive, exhibited an increased amount of searching behavior, and seemed very hungry for the first 15 to 30 minutes. A few animals that were allowed to sleep on larger platforms showed an increase in the length and frequency of D-state in a manner similar to that noted in man.

This study defines a consequence of prolonged D-state and sleep deprivation on levels of brain acetylcholine. The magnitude of decrease in levels of telencephalic acetylcholine in the rat after sleep deprivation has been observed in these laboratories only with psychoactive anticholinergic agents (*14*). This is of interest with respect to the behavioral significance of telencephalic acetylcholine, since the syndrome of sleep deprivation in man consists of many behavioral characteristics also observed with these drugs—such as loss of memory and transient confusion. Methods have yet to be developed to determine whether such symptoms in the sleep-deprived human are related to changes in acetylcholine.

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Hemoglobins of Early Human Embryonic Development

In their report on the predominance of the embryonic hemoglobin Gower 1 in human fetuses (16- to 21-mm crown-rump length), Hecht *et al.* (*1*) wrote that hemoglobin with the electrophoretic mobility of hemoglobin A appeared to constitute at least 10 percent of the total hemoglobin. This concentration of hemoglobin A is unexpectedly high for humans at this stage of development, in view of the finding that the proportion of hemoglobin A is about 8 percent after a 35-week gestation period. Small proportions of hemoglobin A had been noted in the initial report on human embryonic hemoglobins, in embryos with a crown-rump length of 25 to 63 mm (*2*).

In 13 human fetuses with crown-rump length of 15 to 80 mm, we corroborated the findings of Huehns *et al.* (*2*) and Hecht *et al.* (*1*) concerning the presence of hemoglobins Gower 2, Gower 1, F, γ_4, and of a component with the approximate electrophoretic mobility of hemoglobin A. Comparisons on starch gel, in a discontinuous tris–ethylenediaminetetraacetate–boric acid and barbital buffer system (*3*) showed that the last-named component was just perceptibly faster than hemoglobin A. Three hemoglobin samples of this series, belonging to embryos with crown-rump lengths of 25, 40, and 50 mm, were subjected to electrophoresis at *p*H 6.2 on agar plates (*4*). This technique, combined with a strong benzidine reagent, is sensitive enough to determine hemoglobin A in a concentration of less than 1 percent. In all

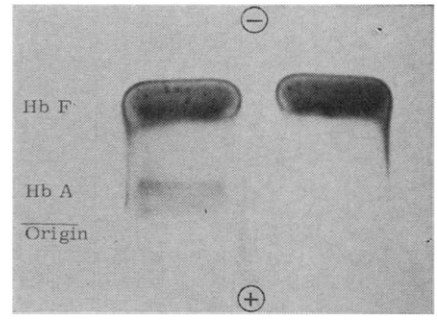

Fig. 1. Agar-gel electrophoresis of hemoglobin, citrate buffer, *p*H 6.2, benzidine stain. Sample shown on left consists of 98 percent hemoglobin F and 2 percent hemoglobin A; the hemoglobin A band is definitely present. Sample on right shows hemolyzate from red cells of a 25-mm embryo; hemoglobin A is absent.