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

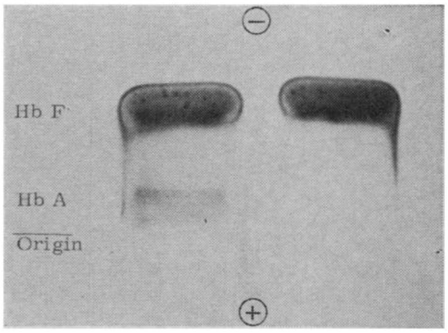

Fig. 1. Agar-gel electrophoresis of hemoglobin, citrate buffer, *p*H 6.2, benzidine stain. Sample shown on left consists of 98 percent hemoglobin F and 2 percent hemoglobin A; the hemoglobin A band is definitely present. Sample on right shows hemolyzate from red cells of a 25-mm embryo; hemoglobin A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

three samples no hemoglobin was detected in the zone corresponding to the adult fractions A and A_2 (Fig. 1). Traces of hemoglobin A could be detected by agar-gel electrophoresis in a fetus of approximately 4 months gestation and 150-mm crown-rump length.

Obviously, the component which moves on starch gel like hemoglobin A is due to a different hemoglobin. At this moment we can offer no further data on the nature of this fraction. Starch-gel electrophoresis may be insufficient to distinguish clearly hemoglobin A from certain other hemoglobins, especially some fetal components. As in the present instance, agar-gel electrophoresis may then be of particular value.

Furthermore, the methods of alkali denaturation or hemoglobin elution are relatively insensitive for identifying small amounts of nonfetal hemoglobin; thus the question of the exact time at which synthesis of β-chains begins remains open. It appears, however, that no β-chains are formed in small embryos whose crown-rump length is less than 100 mm (*6*).

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Hot Shadows on Jupiter

R. L. Wildey has reported a series of intriguing observations in which shadows cast on Jupiter by its satellites have been found hotter at times than their surroundings (*1*). The enigma has been attributed to complicated processes resulting in greater atmospheric transparency, to chemical reactions, and even to magnetohydrodynamics. I feel that there is a much simpler explanation based on a familiar phenomenon.

A clean liquid may be cooled, with care, considerably below its normal freezing temperature. When the liquid finally solidifies the temperature rises abruptly, and it may reach the melting point. The clouds on Jupiter are believed to consist of ammonia, for their infrared reflection spectra do not match ice clouds. The normal freezing temperature of ammonia is 195.5°K.

The usual temperature of the Jovian clouds is about 128°K. Wildey reports that the hot shadows of Ganymede and Europa reached 184.5°K and 191°K, about what would be expected if the sudden passage of the shadows caused enough momentary cooling to trigger the *freezing* of supercooled ammonia cloud droplets.

The effect would not always be present. For it to occur, there must be an adequate number of supercooled liquid droplets carried to the tops of the Jovian clouds by convection. Presumably each of the cloud bands visible on Jupiter has a convectively ascending edge and a convectively descending edge, and supercooled droplets should be more abundant near the ascending edges. The convective motions might be studied by monitoring the shadow temperatures.

Supercooled water droplets account for another puzzle, the observed equality of the light and dark hemisphere cloud temperatures on Venus (*2*). On the dark side, the heat radiated is supplied by the gradual release of latent heat as cloud water freezes. If Venus had a moon we would no doubt observe that its shadow upon the clouds was also hotter than the surrounding clouds, perhaps more consistently than Wildey has observed the effect on Jupiter.

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W. T. Plummer has offered an interesting physical interpretation of my observation that eclipsed regions of Jupiter's atmosphere radiate more at thermal wavelengths than do their surroundings, and do so by a mechanism dependent on some parameter which varies with time (*1*). In defense of his hypothesis put in familiar terms, it must be noted that the mechanism he offers is physically explicit and not *ad hoc* as have been most of my own (*2*). Nevertheless, what is offered is by no means a panacea, for the following reasons.

1) The usual temperature of the Jovian clouds is not 128°K, but more nearly 170°K, as determinations of rotational excitation temperature in the visible indicate (*3, 4*). The low brightness temperature observed at 10 μ requires that matter exists above the clouds whose optical thickness in the visible is negligible, yet of sufficient opacity at 10 μ that our "vision" at that wavelength only penetrates this outer atmosphere deep enough to record a brightness temperature of 128°K. If we do not succeed in producing a transparency in this upper layer, it makes no difference what transient temperature effects occur in the cloud layer because the radiation transients thus produced will not penetrate this outer layer, whose own thermal response to the transient can only be on a very long time scale. Trafton (*4*) has found that hydrogen, known to be above the cloud layer, has the necessary opacity in the form of rotational and free-free transitions in the field interactions of the pressure-induced dipole of H_2 molecules and the intermolecular potential in H_2-He and H_2-H_2 mixtures. Overlying hydrogen will obscure rises in cloud temperature regardless of differences between kinetic temperature and rotational temperature.

2) I have, in fact, monitored the infrared radiation from the satellite shadows, and the published results (*5*) show that when the phenomenon is absent, it is absent regardless of the position of the shadow on the disk, thus in no way depending on the eclipse taking place on a convectively descending cloud edge, as opposed to an ascending edge.

3) Although ammonia has an absorption band completely enveloping the 8- to 14-μ band, its discrete components are extremely sharp, even in the solid state (*6*), so that a "Rosseland" mean opacity over this band would be very low. In the visible, of course, the ammonia opacity is due to pure scattering, which would probably be negligible at 10 μ. It thus appears that even if ammonia were all we could see at 10 μ,