a suitable range. Reserpine exerted a maximal or near-maximal effect in the absence of bretylium pretreatment when given at about 0·25 mg/kg. After bretylium pretreatment, about four times as much reserpine was required to achieve a comparable effect. In the absence of bretylium pretreatment about 8 mg guanethidine/kg exerted a near-maximal effect. The bretylium-pretreated animals required about 32, or more, mg guanethidine per kg to achieve a similar response.

Kuntzman *et al.*[3] state that the "guanethidine and reserpine appear to release norepinephrine by different mechanisms since bretylium, a strongly basic compound, counteracts guanethidine but not reserpine in releasing norepinephrine." It is clear from Fig. 1 that there are not qualitative differences between the bretylium–reserpine, bretylium–guanethidine interactions. A fourfold increase in the dose of either guanethidine or reserpine is required to overcome the effects of 20 mg bretylium/kg given i.p. daily for 2 days.

In our view the similarity of the dose:response curves of guanethidine and reserpine does not furnish insight into the mode of action of these bases. While it may be true that the mechanisms whereby reserpine and guanethidine effect catecholamine depletion are different, the evidence adduced by Kopin and Gordon,[6, 7] based on tissue norepinephrine release studies, offers much stronger support for this hypothesis than the interaction of these drugs with bretylium.

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Brain amines: Response to physiological stress*

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LEVELS of serotonin and norepinephrine in brain respond reliably and selectively to certain pharmacological agents, but a reliable differential response of both amines to nonpharmacological procedures has not been noted. Consequently, several different stressors were investigated for their effects on both monoamines in the brains of 200-g, male, Sprague-Dawley rats.[1]

Norepinephrine and serotonin were assayed in the same brain by fluorescence spectrometry with the method of Mead and Finger[2] and double blind control procedures were used. Changes found by this method were confirmed by use of other standard methods[3, 4] for each of the amines including bioassay for serotonin.[5] In each stress experiment a minimum of four control and four experimental animals was assayed until more than 50 rats had been studied. There were no differences in brain

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weight attributable to the experimental conditions. The positive findings were consistent from experiment to experiment, carried out over a year and a half.

Significant changes were observed when rats were swum to exhaustion. This required 15 to 30 min of sustained effort in 15° water; when the water was 23°, this required 4 to 6 hr of episodic swimming and floating followed by a sustained and enfeebled struggle against drowning. The pattern of change in amine levels was similar to that observed with the psychotomimetic drugs, LSD-25, mescaline, and psychoactive congeners;[6-8] i.e. levels of serotonin increased on the order of 15% and those of norepinephrine decreased by 20% (Table 1).

TABLE 1. RESPONSE OF AMINES

Procedure	Number of rats		Mean (±S.D.)				Δ (%)
	Control	Expt'l	Control (mμg/g)		Expt'l (mμg/g)		
Serotonin							
LSD-25* (260–1,300 μg/kg)	148	195	530	(47)	624	(57)‡	+18
ALD† (1,600 μg/kg)	15	21	538	(33)	603	(36)‡	+12
15° Swim (15–30 min)	41	46	539	(50)	622	(45)‡	+15
23° Swim (4–6 hr)	34	50	533	(42)	604	(62)	+13
Wheel (6 rpm, 3 hr)	25	31	526	(47)	577	(48)	+10
Norepinephrine							
LSD-25 (520–1,300 μg/kg)	20	38	530	(51)	435	(49)	−18
ALD (1,500 μg/kg)	12	12	490	(41)	417	(39)	−17
15° Swim (15–30 min)	23	29	503	(45)	448	(49)	−11
23° Swim (4–6 hr)	23	38	495	(47)	367	(50)‡	−26
Wheel (6 rpm, 3 hr)	12	12	501	(44)	450	(47)	−10

* LSD-25 = *d*-lysergic acid diethylamide.

† ALD = *d*-1-acetyl-lysergic acid diethylamide.

‡ $P < 0.001$ (based on difference between the means between control and treated rats); for all other procedures, $P < 0.01$. Rats were sacrificed 30 to 120 min after LSD-25, 90 min after ALD, and immediately after removal from each stress procedure. Data are expressed as millimicrograms of free base per gram wet weight.

Serotonin levels in rats swum at 23° showed more variability than did those following the 15° swim. Rats on a 12-inch diameter treadwheel for 3 hr at 6 rpm showed only a 10% change in both amines. Longer periods on the treadwheel (up to 24 hr) led to no significant change.[9] This procedure, while requiring exertion and leading to exhaustion, did not evoke the same pattern of activity that was observed with swimming. The rats on the wheel longer than 3 hr tended to give up and take a free ride even if buffeting was involved.

While the specific pattern of activity and level of activation required to induce these changes is not clear, a cold environment inducing a brief period of sustained hyperactivity and exhaustion evoked the most reliable changes. Fifteen rats immersed in chest-high water at 15° were not required to swim, but the situation evoked marked and uninterrupted hyperactivity, an effort to escape, and exhaustion comparable to that observed in the 15° swim. Changes in amine levels in both situations were similar.

A number of other stressors failed to produce significant changes. Shaved animals exposed to an environment of 4° for 3 to 5 hr were not hyperactive nor did they show changes in brain amines. With exposures for periods longer than 11 hr a not significant trend toward elevation of serotonin and a fall in norepinephrine were seen. Prolonged cold exposure has been reported to produce a fall in norepinephrine.[10] There were no consistent effects after electroshock to the feet; Levi and Maynert reported specific parameters of high-intensity shock which did lead to a fall in norepinephrine.[11] No effects were observed after 72 hr of food and water deprivation nor after anoxia which was induced gradually over 10 min in a nitrogen chamber. Serotonin levels did not change after adrenalectomy or sham surgery.[12]

Although the physiological significance of these changes and the mechanisms mediating them are unclear, the changes in brain amines did correlate with changes in behavioral state (Fig. 1). Rats, upon removal from a cold swim, exhibited sporadic uncoordinated activity and exhaustion. After 2 hr they had begun to sustain a few brief periods of coordinated activity such as grooming, and after 4 to 6 hr they appeared to be normal. The elevation of serotonin was significant ($P < 0.001$) immediately after the removal from the water and levels approached normal in 2 hr. On the other hand, values for norepinephrine remained depressed for 2 hr and approached normal by 6 hr. A similar sequence of changes in both behavior and amines was observed with 14 rats permitted to recover from a 4- to 6-hr swim at 23°; with the longer period of time, norepinephrine values were maximally depressed both immediately after removal from the stress as well as 2 hr later. The response of the monoamines to stress is not directly related to the action of the pituitary–adrenal system since levels of adrenal catecholamines and adrenal weights did not change, and the response of brain amines occurred in hypophysectomized rats exposed to the three effective stressors or injected with LSD-25.

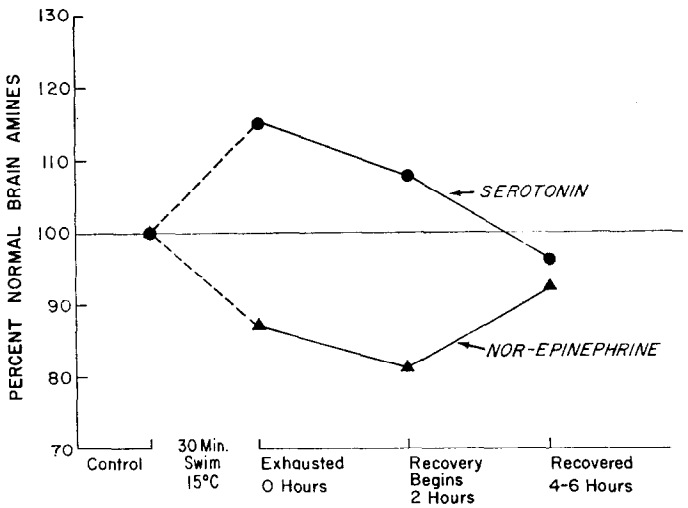

FIG. 1. Behavior and percentage changes of amine levels from normal at intervals after a 30-min, 15° swim are shown. Data are derived from three different experiments involving 52 rats, 26 of which were allowed to recover. For serotonin at 0 hr and norepinephrine at 2 hr, differences between control and experimental groups are significant ($P < 0.001$).

Catecholamines have been implicated in peripheral aspects of temperature regulation.[13–15] In the present experiments the factor of cold, while neither necessary nor sufficient for inducing effects, did influence the rate and extent of change in brain amines. It is of interest that the drugs that induce a similar pattern of change in brain amines produce a unique pattern of central excitation,[16] acting in part on brain mechanisms concerned with the metabolic and physiologic control of body temperature. If the stressors have such a central action, a role for the biogenic amines in central as well as in peripheral aspects of temperature regulation should be sought.

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Further effects of chlorpromazine on the human erythrocyte membrane

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IN PREVIOUS publications[1–3] the authors have demonstrated the ability of phenothiazine derivatives to affect the osmotic permeability of erythrocytes. Further, the alterations in permeability described were obtained with low drug concentrations and correlated well with the clinical potency of these compounds as tranquilizers. The evidence now presented is intended to implicate passive osmotic permeability in the mechanism of action for the phenothiazines.

Erythrocytes were exposed to hypotonic saline, and after centrifugation the supernatants were removed and analyzed for the percentage of hemolyzed cells as previously described.[3] The remaining erythrocytes were analyzed for intracellular ions by a modification of the method of Post and Jolly[4] in the following manner. Cells were suspended in 110 mM magnesium chloride plus sufficient magnesium hydroxide to bring the pH of the wash fluid to 7·4, and then centrifuged at 3000 rev/min in the International clinical centrifuge for 10 min. After discarding the supernatant, the cells were resuspended in fresh magnesium wash fluid and again spun down. The same procedure was repeated a third time. After the final supernatant was decanted, a hemolytic solution consisting of 0·2% (v/v) concentrated ammonium hydroxide reagent plus 0·02% (v/v) Triton X-100 was added to the cells to make a final volume of 10 ml in a volumetric flask. A portion of the hemolysate was analyzed for sodium and potassium with the Baird atomic flame photometer, model KY.[5] A separate portion of hemolysate was added to an equal amount of cyanmethemoglobin reagent (Ortho Pharmaceutical Co., Raritan, N.J.). The concentration of cyanmethemoglobin was then determined by recording the optical density at 540 mμ with the Beckman DU spectrophotometer. The hemoglobin concentration was then read from a standard curve constructed by comparing readings of the unknown with those of known cyanmethemoglobin standards (Ortho). Results were expressed as mEq ion/5 mmoles hemoglobin. This method of expression was chosen because changes in erythrocyte volume which may take place during the experiments do not introduce error into the determinations. Similarly, a correction for interstitial fluid is not required, and the figures obtained are comparable to those presented by the usual method of expression—i.e. milliequivalents of ion per liter of cells. It should