typic set, *F*. Six samples of strains designated S were examined and all were very similar among themselves and to the strains in GL, phenotype set *A*. Table 2 shows several E, W, and S strains compared to GL strains of phenotype sets *A*, *B*, and *C*. Most of the strains are included in set *A*, but several strains belong to set *B* or *C*. Note that comparisons between strains of different sets show very little similarity.

Several explanations for the described situation can be entertained. Perhaps the strains of *Tetrahymena* under laboratory culture undergo very rapid molecular change, so that in a relatively short period of time (two to five decades) two sublines of a strain may become demonstrably different in most of their proteins examined. A second possibility is that controlling loci remain on or off in quasi-stable ways; each strain might be expressing only a fraction of its potential enzyme activity at any one time. Alternatively, the strains so different in their molecular properties did not have a recent common origin, but have inadvertently acquired labels that misrepresent their origins. Unprecedented molecular plasticity is rendered unlikely by the observation that two GL strains, supposedly separated for over 25 years, are virtually indistinguishable. Strain GL-8 was obtained from the laboratory of E. Zeuthen, who obtained his strain from G. W. Kidder in 1947; Kidder's strain is represented by GL-7 (obtained by our laboratory in 1972). These two strains differ by a single variation in one of the seven esterase bands. Moreover, the detailed identity of some of GL, E, W, and S strains would require an unusual convergent evolution and is scarcely explained by random molecular drift. Our provisional interpretation is that the six phenotypic sets represent six collections of wild cells. The variation within sets represents either latent variations in the original isolates (as in a heterozygous state or a macronuclear mosaic), the variations due to control processes, or else mutations which have been established during laboratory culture. The decision as to which phenotypic set corresponds to which original isolate is more difficult to adjudicate.

Regardless of the means whereby they have arrived at their present status, the classical strains of *Tetrahymena* do not constitute a molecularly closely similar set. If the variability of supposedly identical strains (which are used for so many different kinds of biological investigations) is as great as indicated by their electrophoretic mobility patterns, then this variation must be taken into account when comparing the data and conclusions derived from different laboratories. We are depositing two strains of each phenoset in the American Type Culture Collection, Rockville, Maryland, and the Culture Centre of Algae and Protozoa, Cambridge, England, to facilitate the availability of these strains.

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Cyclic Adenosine Monophosphate, Metabolites, and Phosphorylase in Neural Tissue: A Comparison of Methods of Fixation

Abstract. *Fixation of rat brain tissue by freeze-blowing, microwave irradiation, immersion of whole rats in liquid nitrogen, and decapitation into liquid nitrogen indicates that postmortem changes in metabolites and enzyme forms are minimal in freeze-blown brains. Cyclic adenosine monophosphate levels are lowest in microwave-irradiated brains, which has been interpreted by some investigators to indicate rapid fixation and minimal anoxia. However, the changes in phosphocreatine, adenosine triphosphate, lactate, and phosphorylase clearly demonstrate that fixation by freeze-blowing or immersion in liquid nitrogen more closely approximate the state in vivo.*

Preparation of tissue for the study of metabolite levels, transformation of enzyme forms, and cofactors involved in metabolic regulation demands that metabolic processes be stopped as quickly as possible. The enormously high metabolic rate of the brain coupled with its limited energy reserves means that substantial changes can take place if the fixation is not sufficiently rapid.

Recently, two new techniques have been developed, one of freeze-blowing the brain and another of fixation by microwave irradiation. The freeze-blowing of the brain fixes the tissue in less than 1 second and has been shown to preserve the energy reserves of the brain better than immersion in liquid nitrogen or microwave irradiation (*1*, *2*). However, this technique precludes the use of brain tissue for regional studies. In another study, microwave irradiation was used to fix the brain for the measurement of cyclic adenosine monophosphate (cyclic AMP) levels (*3*). The results indicated that cyclic AMP concentrations were in good agreement with measurements made by quick-freezing and offered the possibility of regional studies. However, total inactivation of the enzymes involved in cyclic AMP synthesis and degradation required at least 20 seconds of exposure (*4*), suggesting that changes in some metabolites or forms of enzymes or both might occur. After irradiation of mouse heads 1 second after decapitation, Nelson and Mantz (*5*) found that there was a differential effect on the enzymes of glycolysis resulting in low levels of lactate but breakdown of glycogen and adenosine triphosphate (ATP). In the present report, substances which are sensitive indicators of the degree of anoxia or ischemia or both in the brain have been measured in rat brains which have been fixed by immersion in liquid nitrogen, freeze-blowing, or microwave irradiation. The levels of phosphocreatine, lactate, ATP, cyclic AMP, and the conversion of phosphorylase *b* to *a* have been studied.

Male Wistar rats (Carworth Farms) weighing 200 to 250 g were starved overnight prior to use. Fixation of brain tissue was accomplished in the following ways: (i) the whole animal was immersed in liquid nitrogen for 5

Table 1. Concentrations of cyclic AMP and metabolites, and phosphorylase *a* as a percentage of total phosphorylase, in rat brain following various methods of fixation. The rats were killed, their brains extracted, and analyses were performed as described in the text. Values represent the mean and the standard error of the mean.

Method*	Cyclic AMP (μmole/kg)†	Phosphocreatine (mmole/kg)†	ATP (mmole/kg)†	Lactate (mmole/kg)†	Phosphorylase *a* (%)
Freeze-blown (9)	1.17 ± 0.13	4.00 ± 0.10	2.41 ± 0.08	1.23 ± 0.07	20.1 ± 0.69
Microwave irradiation (9)	0.73 ± 0.05‡	1.72 ± 0.08‡	1.68 ± 0.03‡	1.71 ± 0.15‡	
Immersion into liquid N_2 (6)	1.63 ± 0.16‡	3.40 ± 0.22	2.30 ± 0.07	1.90 ± 0.11‡	53.9 ± 4.8‡
Decapitation into liquid N_2 (5)	2.62 ± 0.17‡	1.41 ± 0.03‡	1.79 ± 0.06‡	3.16 ± 0.13‡	62.3 ± 8.3‡

* Number of rats per group is given in parentheses. † Wet weight. ‡ Difference from freeze-blown values is statistically significant with $P < .005$.

minutes, (ii) the severed heads were frozen in liquid nitrogen for 3 minutes, (iii) the brains were freeze-blown as described by Veech *et al.* (*1*, *2*), or (iv) the animals were irradiated in a model Mc-24 microwave oven according to the method of Medina [see (*2*)]. In the freeze-blowing method, two probes are driven simultaneously into the cranial vault of the rat. Air pressure (25 pounds per square inch; 1.7 atm) enters through one probe and blows the supratentorial brain tissue into a metal chamber cooled in liquid nitrogen. The microwave oven had a 1500-watt, Dx-206 magnetron operating at a frequency of 2450 Mhz and was modified to deliver 1100 to 1200 watts directly to the rat head through a metal wave guide. The intact brains were dissected at −20°C and all samples were extracted with perchloric acid as described by Nelson *et al.* (*6*). Cyclic AMP was assayed according to the method of Gilman (*7*). The remaining measurements were made by the methods of Lowry *et al.* as follows: phosphorylase (*8*), and ATP, phosphocreatine, and lactate (*9*).

Measurements of five substances were used to compare the relative efficacy of the four different fixation techniques (Table 1). Phosphocreatine is one of the best indicators of energy status of the brain since it is most rapidly utilized during ischemia (*10*). The phosphocreatine levels are highest in the freeze-blown brain, while in the brain frozen by immersion the levels are somewhat lower. In the brains fixed by microwave irradiation or decapitation into liquid nitrogen, the phosphocreatine levels are 43 and 35 percent, respectively, of the concentration in the freeze-blown brain. Adenosine triphosphate, which is sustained for a longer period of time than phosphocreatine during ischemia, is not different in the freeze-blown brains and in the brains of immersed animals. However, ATP was significantly reduced to 70 percent of the highest levels in the brains of decapitated animals, and to 66 percent in the irradiated brains. Lactate concentrations which rise rapidly during ischemia (*10*) are lowest in the freeze-blown brains and highest in the brains of the decapitated animals.

Phosphorylase has been shown to be primarily in the *b* form if the brains are fixed rapidly (*11*). The portion of phosphorylase in the *a* or active form is 20 percent in freeze-blown brains and is 54 and 62 percent in the immersed and decapitated animals, respectively. Cyclic AMP concentrations have been shown to increase in the brain during anoxia produced by decapitation (*12*). The lowest levels are found in the irradiated brain, with progressively increasing amounts in brains which are freeze-blown, frozen by immersion, and frozen after decapitation.

By criteria applied to assess the metabolic status in brain, the freeze-blown brain appears to approximate the in vivo situation most closely. The differences in the energy reserves and lactate between freeze-blown brains and brains fixed by other means can be used as a measure of high-energy phosphate (~ P) use during the other methods of fixation, which together with the metabolic rate of brain can be used to calculate the time required for fixation. The energy use can be calculated from the formula: $\sim P = \Delta$ phosphocreatine $+ 2 \times \Delta$ ATP $+ \Delta$ lactate (*10*). The sums of the differences from freeze-blown brains, indicating ~ P use (millimoles per kilogram) for the various methods of fixation are as follows: 0.97 for the brains of the immersed animals, 4.22 for the microwave-irradiated brains, and 5.76 for the brains of the decapitated animals. The metabolic rate of brain has been found to be approximately 20 mmole of ~ P per kilogram per minute (*13*). The ~ P use divided by the metabolic rate shows that 2.9, 12.6, and 17.3 seconds are required for effective fixation of the brains of immersed, irradiated, and decapitated animals, respectively.

The lack of increase in cyclic AMP concentration in the irradiated brain in spite of the evidence of significant anoxia dissociates these two events in the microwave-irradiated brain. A possible explanation is that the adenyl cyclase and phosphodiesterase are affected by the heat in such a manner that any increase in cyclic AMP due to anoxia is masked by its immediate degradation by phosphodiesterase. A second possibility is that the heat during irradiation affects the cyclic AMP binding to protein kinase. Increases in temperature favor the dissociation from bound to free cyclic AMP, which is then available as substrate for phosphodiesterase (*14*). Hydrolysis of the free cyclic AMP by phosphodiesterase could thus account for the low levels in irradiated brains. A further possibility might be the lack of stimulation of cyclic AMP formation by neurotransmitters. The high level of acetylcholine found in brains fixed by microwave irradiation (*15*) might result from the prevention of the release of neurotransmitter. If norepinephrine, for example, were similarly affected, the increase in cyclic AMP due to this agent could be blocked.

The method of fixation of brain tissue is clearly dictated by the objectives. The measurement of metabolite concentrations and enzyme activities is better accomplished in frozen brains. Although frozen whole brains are difficult to dissect, the frozen tissue is also preferable for regional studies of superficial layers of the brain. Although it has been shown that deeper layers of the brain freeze more slowly (*16*), it is possible to evaluate the time of freezing and consequent changes (*17*). The intense heat generated by microwave irradiation disrupts cell membranes, permitting diffusion of substances (*18*). As long as the brain is kept frozen at low temperatures diffusion is minimized. If metabolites and metabolic events associated with cyclic AMP concentration are to be investigated under conditions in which the

in vivo energy status is maintained, optimal fixation is accomplished by freeze-blowing the brain.

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Objective Assessment of Hypnotically Induced Time Distortion

Abstract. *The objective precision of operant conditioning methodology validates the power of hypnosis to induce alterations in time perception. Personal tempo was systematically modified by instructions to trained hypnotic subjects, with significant behavioral effects observed on a variety of response rate measures.*

Time perception is one of the most important, although least studied, consequences of the socialization process. Infants and children, whose behavior is primarily under the control of biological and situational exigencies, must be taught to develop a temporal perspective in which the immediacy of the experienced reality of the present is constrained by the hypothetical constructs of past and future. Society thereby transforms idiosyncratic, impulsive, and potentially disruptive behavior into approved, predictable, controllable reactions through the time-bound mechanisms of responsibility, obligation, guilt, incentive, and delayed gratification (*1*). The social acceptability of such reactions often depends on their rate of emission as much as upon other qualitative aspects. Thus, we develop, in addition to a sense of temporal perspective, a time sense of personal tempo, which involves both the estimation of the rate at which events are (or should be) occurring and affective reactions to different rates of stimulus input (*2*).

The learned correspondence between our subjective time sense and objective clock time can be disrupted by the physiological and psychological changes that accompany some types of mental illness, emotional arousal, body temperature variations, and drug-induced reactions (*3*). However, it is possible to modify either temporal perspective or tempo within a controlled experimental paradigm by means of hypnosis. Our previous research demonstrates the marked changes in cognition, affect, and action that result when hypnotized subjects internalize the instruction to experience a sense of "expanded present" (*4*). However, the data used to document such changes in this and related studies (*5*) have been too subjective and gross. In the present study we attempted to alter personal tempo and measure the behavioral consequences with precise, objective techniques.

The experience of tempo was systematically varied (speeded up or slowed down) by time-distorting instructions administered to hypnotic subjects and controls. If effective, such a manipulation should generate asynchronicity between clock time and the subjective passage of time. This asynchronous responding was assessed by means of the objective precision of a specially designed operant conditioning and recording apparatus. As predicted, the operant behavior of these hypnotized subjects was significantly altered relative to their own normal baseline and also to that of subjects in two control conditions.

The volunteer subjects were 36 Stanford University undergraduates of both sexes, who were selected from among the high scorers on a modified version of the Harvard group scale of hypnotic susceptibility (*6*) administered in their introductory psychology class. They were each randomly assigned to one of three treatments: hypnosis, hypnotic role-playing, and waking nonhypnotized controls. Before the experiment, the hypnosis group underwent a 10-hour training program designed to teach them to relax deeply; to concentrate; to experience distortions in perception, memory, and causal attribution; and to induce autohypnosis. The other subjects received no prior training. During the experiment, the testing procedure was identical for all subjects; an experimenter who was unaware of the experimental treatment delivered the standardized instructions to the subject, who sat isolated in an acoustic chamber. A second experimenter induced a state of hypnotic relaxation in the hypnosis group and instructed the hypnotic role-playing subjects to try their best to simulate the reactions of hypnotic subjects, to behave as if they were really hypnotized throughout the study. The waking controls were told only to relax for a period of time equivalent to that given to subjects in the other two treatments.

Subjects were taught to press a telegraph key at different rates in order to illuminate various target lights in an array of ten colored lights. In the first of five 2-minute trials, a comfortable operant rate of responding was established, and it became obvious to the

Table 1. Tempo modification. Data are mean deviations in the rate from baseline performance.

Treatment	*N*	No feedback	Objective feedback	Combined
Hypnotized	12	.534	.233	.38*
Role players	12	.299	.004	.15†
Waking controls	12	.023	.043	.03
		$P < .025$	$P < .005$	$P < .001$

* $P < .01$ for comparison with role players; $P < .001$ for comparison with waking controls. † Comparison with waking controls not significant.