increase in SCE frequency at average levels of exposure that are low in comparison with the current OSHA standard of 50 ppm. With the breathing zone data gathered in this study, it may be possible to determine whether the observed increase in SCE's arises exclusively from the cumulative effect of daily exposure or whether some component of the increase results from the rate at which that exposure occurs. Comparison of mean numbers of SCE's induced per cell per unit of cumulative exposure with those reported in a recent animal study (*6*) indicates that humans may be considerably more sensitive to SCE induction than animals.

This difference would be much less, however, if SCE induction were also a function of dose rate, since the workers were exposed to ETO for short periods at five times the dose rate to which the animals were exposed. An effect of dose rate has been shown for ETO in other animal studies (*12*). If a dose rate effect is found for humans as well, then the evidence of ETO-induced SCE's may suggest that occupational exposure to ETO and other alkylating agents be controlled in terms of both cumulative dose and dose rate.

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10. The Miran 1A infrared spectrophotometer has a rapid response time and is sensitive to 1 ppm ETO. It was linked to a strip-chart recorder with integral output to continuously monitor air concentrations during the defined tasks. Five-foot-long tubing with a sampling probe was attached to the instrument and hand-held in the breathing zone of the worker while the specified tasks were performed. The instrument was calibrated at the recommended wavelength of 3.3 μm for the mixture used at hospital A (12 percent ETO and 88 percent Freon 12) and at 11.8 μm for the 100 percent ETO at hospital B. Thirty measurements were obtained for the task that contributed most to exposure (task 1 at hospital A) (Table 1). Statistical testing showed no detectable difference between the assumptions of a normal or a log-normal distribution for the samples. The 8-hour TWA was calculated to be 0.92 ppm (range, 0.51 to 1.26 ppm).
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In vivo Phosphorus-31 Nuclear Magnetic Resonance Reveals Lowered ATP During Heat Shock of *Tetrahymena*

Abstract. *Cells synthesize a characteristic set of proteins—heat shock proteins—in response to a rapid temperature jump or certain other stress treatments. The technique of phosphorus-31 nuclear magnetic resonance spectroscopy was used to examine in vivo the effects of temperature jump on two species of* Tetrahymena *that initiate the heat shock response at different temperatures. An immediate 50 percent decrease in cellular adenosine triphosphate was observed when either species was jumped to a temperature that strongly induces synthesis of heat shock proteins. This new adenosine triphosphate concentration was maintained at the heat shock temperature.*

Cells respond in a common manner to a rapid temperature jump. This heat shock response involves the immediate transcriptional activation of a small set of previously quiescent genes, the transcripts of which are then preferentially translated into a characteristic set of proteins, the heat shock proteins. Conversely, both transcription and translation of most non–heat shock genes and transcripts are greatly reduced at the elevated temperature (*1*). The synthesis of these heat shock proteins, especially a class with a molecular weight of approximately 70,000, has been strongly conserved during evolution, and their presence is diagnostic of the response. In addition to temperature jump, these proteins are synthesized by cells in response to a variety of other stresses, including treatment with respiratory inhibitors, amino acid analogs, release from anoxia, and, in the case of *Tetrahymena*, deciliation (*1, 2*). The functions of this general cellular response to stress and of the induced proteins are not known, but their uniform occurrence in diverse organisms suggests that they play a fundamental role in cellular homeostasis.

The physiological signals mediating the heat shock response are unknown. However, because many inhibitors of oxidative phosphorylation or electron transport also induce synthesis of heat shock proteins, it has been suggested that cellular adenosine triphosphate (ATP) may be involved in the response (*3*). We used ^{31}P nuclear magnetic resonance (NMR) spectroscopy to examine in vivo the effects of temperature jump on the ciliated protozoan *Tetrahymena*. We compared two species of *Tetrahymena* in which the response is induced by different temperatures.

Cells in logarithmic growth were concentrated by centrifugation, washed, and resuspended at high density. The ^{31}P NMR spectra of the cells maintained at a standard temperature were collected, and the cells were then subjected to a temperature jump and further spectra were obtained. Portions were removed at both temperatures, and protein synthesis was monitored in vivo by incubating the cells in the presence of tritiated amino acids at either temperature. The ^{3}H-labeled proteins were then identified by gel electrophoresis and fluorography (*4*).

Upon subjecting either species to the higher temperature, where heat shock

proteins are synthesized, we observed an immediate 50 percent decrease in cellular ATP concentration. As shown in Fig. 1A, jumping suspensions of *T. thermophila* from 30° to 40°C induced synthesis of the heat shock proteins. The NMR spectra recorded simultaneously from a sample of the same cells showed a decrease in intracellular ATP from 3.3 ± 0.3 m*M* to 1.6 ± 0.6 m*M* ($N = 9$) at the higher temperature (Fig. 1A). No change in cell viability was observed after heat shock.

A similar experiment was conducted with *T. pyriformis*, in which the heat shock response is induced by a temperature jump from 28° to 33°C. As seen in Fig. 1B, this temperature jump induced synthesis of the heat shock proteins and was also accompanied by a 50 percent decrease in ATP concentration. These similar decreases in cellular ATP were observed at 33° or 40°C in the two species, suggesting that this change in ATP concentration is not simply a function of temperature but represents a fundamental aspect of the cellular response to heat stress.

The change in ATP was detected within 3 minutes after raising the temperature, which is near the limit of time resolution in our NMR experiment. After this initial decrease, ATP did not continue to decline during prolonged exposure to the elevated temperature but stabilized at a new steady-state level. However, this new level depended on the severity of the heat shock. When *T. thermophila* was jumped to 37°C instead of 40°C, only a 25 percent decline in ATP was observed and heat shock protein synthesis was less intense than at 40°C, as determined by inspection of gel fluorograms.

The possibility that ATP concentrations change after heat shock has been considered previously, since it has been known for some time that many inhibitors of respiration also induce the response. However, attempts to correlate these treatments with ATP levels in cell lysates of *Drosophila* salivary glands resulted in conflicting measurements (*1*, *3*). Interestingly, a decrease in cellular ATP was noted in *Tetrahymena* lysates after the cells were subjected to a series of heat treatments, traditionally used as a method of synchronization (*5*). In all these instances, however, ATP levels were determined with cell lysates, and the possibility of hydrolysis of ATP during preparation cannot be excluded (*6*). This possibility is eliminated in our resolution of cellular ATP levels in vivo.

No accompanying increases in adenosine diphosphate (ADP) or adenosine monophosphate (AMP) were observed along with the decrease in ATP. If ATP were hydrolyzed to ADP, we would observe no change in the peak at −10 ppm, which is composed of resonances from the α phosphates of ADP and ATP.

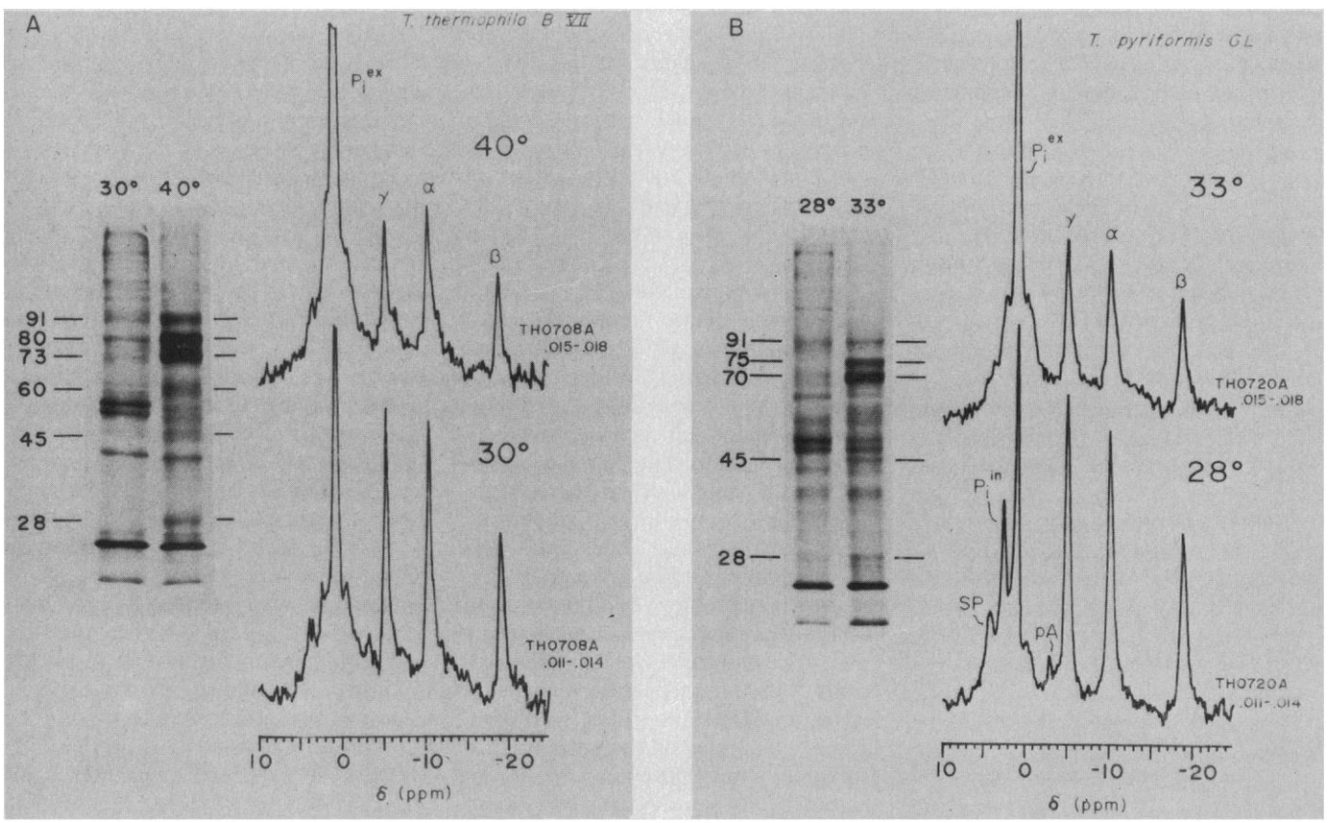

Fig. 1. Protein synthesis and ^{31}P NMR spectra in control and heat-shocked *Tetrahymena*. (A) Fluorogram of sodium dodecyl sulfate (SDS) gel showing proteins synthesized and ^{31}P NMR spectra of *T. thermophila* immediately before (30°C) and after (40°C) heat shock treatment. Portions of cell samples used to obtain NMR spectra were labeled in the presence of ^{3}H-labeled amino acids, and whole cell protein was extracted and analyzed on SDS acrylamide gels. Labeled proteins were visualized by fluorography. Note the appearance of characteristic heat shock proteins with molecular weights of 91,000, 80,000, 73,000, 60,000, 45,000, and 28,000 and the decline in synthesis of other proteins at 40°C. The ^{31}P NMR spectra represent approximately 30 minutes of accumulation (1200 free induction decays each). Note the decline in ATP, in this case from 3.6 m*M* before shock to 1.3 m*M* after shock. Because of the high level of external phosphate (P_i^{ex}) in this sample (4 m*M*), the intracellular *p*H was not accurately determined. (B) Fluorogram and ^{31}P NMR spectra of *T. pyriformis* immediately before (28°C) and after (33°C) heat shock treatment. Note again the appearance of heat shock proteins at molecular weights of 91,000, 75,000, 70,000, 45,000, and 28,000. Differences in molecular weights of the heat shock proteins are consistently observed between the two species (*11*). As in (A), ^{31}P NMR spectra show a decline of cellular ATP from 3.4 to 1.6 m*M*. In this sample the intracellular phosphate (P_i^{in}) resonance is clearly resolved at 28°C but shifts upfield after heat shock, indicating intracellular acidification. Abbreviations: *SP*, sugar phosphates; *pA*; phosphoarginine; α, β, γ, primary, middle, and terminal phosphates of ATP, respectively. (The α and β resonances of ADP phosphates coresonate with α and γ phosphates of ATP, respectively.)

Figure 1, A and B, clearly shows a decrease in the intensity of this peak, indicating that no significant increases in ADP occurred. If present, AMP can generally be well resolved at around +4 ppm, yet no major resonance was detected in this range. Concomitant with the loss of ATP, there was a dramatic increase in the intensity of the inorganic phosphate (P_i) peak, indicating that ATP was hydrolyzed and not simply sequestered or precipitated (*7*). These observations suggest that ATP is hydrolyzed to the free nucleoside, which is not observed by ^{31}P NMR.

Intracellular *p*H was also observed to collapse after heat shock (Fig. 1). The *p*H decreased less rapidly than the ATP concentration and reached its final value over a period of approximately 10 minutes. At an external *p*H of 6.2, the intracellular *p*H, which is usually about 7.2, shifted to approximately 6.7 upon heat shock ($N = 3$). When the external *p*H was adjusted to 7.2, the intra- and extracellular phosphate peaks coincided at +2.7 ppm. If there were intracellular acidification after heat shock and no decrease in internal P_i, we would expect to observe the appearance of an upfield resonance (at about +2.1 ppm). This did not occur, indicating that the intracellular medium was not acidified by more than 0.2 *p*H unit upon heat shock at the higher external *p*H. These results suggest that upon heat shock and loss of cellular ATP, cells become less able to maintain an alkaline *p*H gradient. They also suggest that lowered intracellular *p*H is not a condition for the heat shock response.

These observations indicate that the changes in cellular physiology associated with heat shock are more dramatic than was previously realized. We detected significant decreases in the steady-state levels of cellular ATP within 3 minutes after heat shock, which are well correlated with the existence and extent of heat shock protein synthesis. Like the transcriptional activation of the heat shock genes, these changes occur essentially immediately upon initiation of heat shock. It will be of interest to determine whether these changes in ATP are involved in triggering the general cellular response to heat shock.

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4. Cells were grown to mid-logarithmic phase (2×10^5 cell/ml) in 2 percent proteose peptone, 0.1 percent yeast extract, 0.2 percent glucose, and 0.003 percent sequestrin at either 28°C (*T. pyriformis*) or 30°C (*T. thermophila*) and were harvested by gentle centrifugation. The pellets were washed and resuspended at 7×10^6 cell/ml in 20 m*M* 2-(*N*-morpholino)ethanesulfonate, 1.0 m*M* sodium phosphate, *p*H 6.0. This cell density represents an approximately 25 percent (by volume) cell suspension, which corresponds to an intracellular : extracellular volume ratio of 10 percent. Concentrated cultures (30 ml) were maintained for up to 6 hours with shaking at 250 rev/min in 250-ml culture flasks. At the beginning of each experiment, 14-ml samples were placed in 20-mm NMR tubes capped with an aeration manifold through which a mixture of 95 percent O_2 and 5 percent CO_2 was bubbled at a rate that maintains the O_2 tension well above the Michaelis constant K_m for O_2 uptake (*8*). Samples were placed in a Bruker WH-360-WB NMR spectrometer operating at 145.78 MHz in pulsed Fourier-transform mode. Spectra were accumulated and stored approximately every 7.5 minutes as the sum of 300 free induction decays arising from 45° tipping pulses applied every 1.5 seconds. In some cases, different acquisition parameters were used to rule out possible effects of temperature on the spin-lattice relaxation time (T_1) that could lead to erroneous concentration values. The ATP levels were quantified by comparison with the known external phosphate concentration, correcting for both saturation differences and the intracellular : extracellular volume ratio. The *p*H was calculated from the chemical shifts of the P_i peaks (*8*).

   After 1 hour of spectrum accumulation at standard temperatures, samples were withdrawn from the magnet and 0.4 ml was removed for protein labeling. The NMR sample was then brought to 33°C (*T. pyriformis*) or 40°C (*T. thermophila*) within 30 seconds by briefly immersing the spectrometer tube in a water bath at 55°C and then in a second bath equilibrated at the final heat shock temperature. During this time, the temperature of the NMR probe was also increased to the heat shock temperature. Samples were returned to the probe within 2 minutes of the heat shock. After one spectrum was acquired at the higher temperature (5 minutes), the sample was again withdrawn from the magnet, the temperature checked, and a second 0.4-ml portion removed for protein labeling. The sample was then returned to the magnet to continue spectrum accumulation.

   Portions removed for labeling of proteins were incubated with oxygenation at the control or the heat shock temperature. These portions contained 2.5×10^6 cells and were labeled in the presence of 75 μCi/ml of high specific activity ^3H-labeled amino acid mixture (Amersham) for 1 hour. Whole cell protein was prepared as described in (*2*), except that 8*M* urea was added to the lysis buffer, which was heated to 100°C. Equal volumes of lysate from approximately 1×10^5 cells were analyzed on a 10-cm, 10 percent SDS acrylamide gel (*9*), and were then processed for fluorography (*10*).
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Infection of Normal Human Epithelial Cells by Epstein-Barr Virus

Abstract. *Primary cultures of epithelial cells were grown from the tonsils and adenoids of patients with diseases not related to Epstein-Barr virus. The cells could not be infected by Epstein-Barr virus. Fluorescein-labeled Epstein-Barr virus and a cytofluorograph were then used to show that the epithelial cells do not have detectable receptors for the virus. However, implantation with Epstein-Barr virus receptors gave the cells the ability to bind the labeled virus. One to 5 percent of receptor-implanted cells exposed to the transforming B95-8 substrain of the virus expressed Epstein-Barr nuclear antigen. The early and viral capsid Epstein-Barr virus–determined antigens were not detected in the virus-infected cultures. The results show that normal human epithelial cells from the nasopharynx become susceptible to infection by Epstein-Barr virus when the membrane barrier resulting from the lack of viral receptors is overcome by receptor implantation.*

Nasopharyngeal carcinoma occurs mainly among Cantonese Chinese, Alaskan natives, and in people in some regions of northern and equatorial Africa. The disease is common among adults and can occur in children and adolescents as well (*1*). The undifferentiated histophathologic type of nasopharyngeal carcinoma is consistently associated with Epstein-Barr virus (EBV), a lymphotropic human herpesvirus that causes infectious mononucleosis and is implicated in the genesis of Burkitt's lymphoma (*2*). Epstein-Barr virus DNA and Epstein-Barr nuclear antigen (EBNA) can be demonstrated in biopsy specimens of nasopharyngeal carcinoma (*3*). Epithelial cells, but not the lymphocytes