

References and Notes

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- Abbreviations are as follows: sRNA, soluble RNA; mRNA, messenger RNA; ATP, adenosine triphosphate; GTP, guanosine triphosphate; polyUG, poly(uridylic-guanylic) acid; polyAUG, poly(adenylic-uridylic-guanylic) acid; A, adenosine; U, uridine; C, cytidine; G, guanosine; TMV, tobacco mosaic virus; TYMV, turnip yellow mosaic virus; TCA, trichloroacetic acid. In the abbreviations for trinucleotides and polynucleotides, the first letter stands for the 5' terminus. For example UUU is UpUpU. For amino acids: met, methionine; leu, leucine; ala, alanine; AsN, asparagine; asp, aspartic acid; glu, glutamic acid; pro, proline; arg, arginine; lys, lysine; ser, serine; cys, half cysteine; thr, threonine.
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- After this paper was submitted, the prediction that *N*-formylmethionyl-sRNA functions as chain initiator has been confirmed for bacteriophage R17 (J. Adams and M. Capocchi, Memo No. 121, Information Exchange Group No. 7) and f2 (N. Zinder, personal communication) coat proteins synthesized in an *E. coli* cell-free system. Since the product synthesized in vitro terminates with *N*-formylmethionine and the natural coat protein with NH_2 -alanylserine, the terminal methionine must be removed after the completed polypeptide chain has been released from the ribosome. After this article was submitted, Marcker also suggested that *N*-formylmethionine might be used for chain initiation (K. Marcker, *J. Mol. Biol.* **14**, 63, 1965), providing definitive evidence that attachment of methionine to sRNA and formylation occurs in two steps catalyzed by two different enzymes. Thus, the question remains open how the ambiguous codon UUG within a chain is translated unambiguously, unless the major codon for *N*-formylmethionine is different, for example CUG.
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Energy Balancing in *Nitella* Cells Treated with Dinitrophenol

Abstract. *The toxic action of 2,4-dinitrophenol on the large cells of the alga, Nitella clavata, was evaluated, with the rate of protoplasmic streaming and the survival time at three light intensities used as criteria. At a sufficiently high intensity the cells survived several weeks, an indication that the energy-uncoupling action of dinitrophenol could be counterbalanced to some extent by an increased energy input. In the treated cells chloroplasts moved from the outer gel-type cytoplasm into the inner, streaming cytoplasm.*

The rate of protoplasmic streaming in the large internodal cells of *Nitella clavata* is dependent on the cellular energy supply (light intensity) only at low levels of illumination (I). Moreover, the quantitative relation between streaming rate and light intensity has been demonstrable only in cells kept under constant conditions for several weeks. It can be assumed that energy storage products in the cell had to be depleted before the cell became a stationary system, at least with regard to the flow of energy.

We now report the opposing effects of 2,4-dinitrophenol (DNP) and light on the energy balance of *Nitella*. A light intensity of sufficient magnitude serves to counterbalance the toxic action of $2 \times 10^{-5}M$ dinitrophenol at pH 6.7 (Fig. 1). All cells at the two lower light intensities died. At the highest light intensity (2700 erg $\text{cm}^{-2} \text{sec}^{-1}$, corresponding to about 540 lux, from incandescent lamps) an apparently stationary state is attained within 2 days, with the streaming rate reduced to about 70 percent of the control value.

The quantitative relationship for untreated cells (2) is as follows: at the "bare-survival" light intensity of 120 erg $\text{cm}^{-2} \text{sec}^{-1}$ the streaming rate is 60 percent of the maximum (saturation

value); the streaming rate increases with increasing light intensity until the maximum is attained at 600 erg $\text{cm}^{-2} \text{sec}^{-1}$, remaining at this level for higher intensities. If the relative streaming rate of DNP-treated cells is a valid index of the cellular energy supply, then the cells at 2700 erg $\text{cm}^{-2} \text{sec}^{-1}$ are comparable to untreated cells slightly above

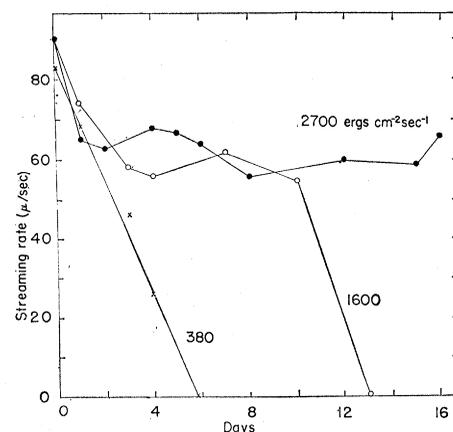


Fig. 1. The influence of light intensity on the rate of protoplasmic streaming and survival time of typical cells of *Nitella clavata* in a solution (7) containing $2 \times 10^{-5}M$ dinitrophenol, pH 6.7. Cells having no streaming are dead. The mean streaming rate for control cells at the two higher light intensities was $89 \pm 3 \mu/\text{sec}$ over the 16-day period. Temperature, 22°C .

the threshold light intensity for survival. By linear interpolation this value is about $240 \text{ erg cm}^{-2} \text{ sec}^{-1}$. On this basis the useful energy obtained by these cells is only about 9 percent of normal.

When the DNP concentration was increased to $5 \times 10^{-5}M$, the mean survival time was independent of the light intensity, being 2 days at each of the three light intensities. Cells kept in the control solution in darkness survived 33 days on the average; this appreciable survival time is attributable to a slow utilization of energy storage products. No estimate of the absolute magnitude of the energy requirement for survival of control cells in light can be made without an assumption regarding the photosynthetic efficiency. About two-thirds of the incident light is absorbed by *Nitella* cells (1).

The way in which DNP reduces the energy available for cellular functions would appear to be through the uncoupling of respiratory-chain phosphorylation or the stimulation of adenosine triphosphate hydrolysis, or both, rather than through the inhibition of photophosphorylation (3, 4). Although DNP can be reduced to 2-amino-4-nitrophenol both by isolated chloroplasts (5) and animal cells (6), the extent of this detoxication process in the present work is not known. In respiratory chain uncoupling, the diversion of metabolic electrons into net DNP reduction is not an important factor, since oxygen uptake remains normal or is even increased (3). We know of no direct effect of light on DNP.

The toxicity of DNP depends on its concentration at the site of action, and thus is related to the rate at which it enters the cell. Passage through the cell membrane appears to be chiefly in the undissociated form (acid dissociation constant, 8×10^{-5}), as no detrimental effects were observed at pH 8.3. For the main results at pH 6.7 and a total DNP concentration of $2 \times 10^{-5}M$, the concentration of undissociated DNP is $5 \times 10^{-8}M$.

An interesting feature of *Nitella* cells surviving under prolonged DNP treatment was the migration of most of the chloroplasts from their stationary positions in the outer, gel-type cytoplasm to the inner, streaming cytoplasm. The release of the chloroplasts from their very regular arrangement in the outer plasm (like bricks in pavement) suggests that DNP may cause a gel-sol trans-

formation of the cytoplasmic gel. Under normal conditions only a few chloroplasts of a total of about 2 million are present in the streaming cytoplasm. The characteristic smoothness of streaming did not appear to be affected by this alteration in the internal arrangement of the cell parts.

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Attention in the Newborn: Effect on Motility and Skin Potential

Abstract. Newborn infants showed lower motility and greater reactivity of the skin potential while attending to a visual target than when equally alert but inattentive.

Many studies demonstrate that the human newborn attends to appropriately presented patterned visual stimuli (1). He can also make some rudimentary perceptual discriminations. Still unanswered are questions regarding the adaptive or regulatory significance of these earliest states of attentive fixation or visual orientation.

Evidence indicates that during the first 10 days of life a baby lying relatively still, with eyes open, typically reacts to the presentation of a patterned visual target with orientation to the target, visual fixation on the target area, widening of the eyes, and reduction of the mild, steady activity of limb and trunk that is usually present in the waking but undistressed state.

By contrast, from about 15 days of age the orientation-quiescence reaction still occurs upon appropriate presentation of a stimulus, but, after some 15 to 30 seconds of quiet looking, certain stimuli will elicit manifest excitation characterized by kicking, squirming, panting, and sometimes smiling and cooing (2). We have attempted more systematic exploration of the nature of this response of the newborn, particularly as it relates to the regulation of motor activity and autonomic reactivity.

Fifteen normal full-term infants between 2 and 6 days of age were observed and tested under two conditions: they were lying in their cribs in a quiet, wakeful state with eyes wide open, either with no target (condition A) or with a vivid visual target of random nonintersecting black lines on a white background presented at a distance of 20 cm in a plane normal to their line of vision (condition B). The stimulus card measured 24 by 27.5 cm; its lines were 6 mm wide (Fig. 1). Room lighting was normal. The sides of the cribs were made of a smooth light-blue plastic material so that under condition A no patterned visual target was available. Each condition was maintained for 1 minute and was alternated with the other condition for a total of 12 trials for each baby, six under each condition. The initial condition for each experimental run was randomly selected.

Approximately 15 seconds after the onset of each condition, and under condition B only when the baby had gazed at the target for at least 10 seconds, a puff of nitrogen was directed at an area just above the umbilicus. The 1-second puff was delivered from a hose held 5 cm above the abdomen under a pressure of 200 g/cm². The jet was just strong enough to slightly indent the skin.

The three dependent variables recorded were motility, skin-potential reaction, and heart-rate reaction. Because of technical failures in the cardiograph, the data on heart rate were not analyzable. Motility was recorded by use of a mattress constructed of polyurethane foam enclosed in an airtight rubber casing. Slight positive pressure was introduced into the casing. Movements of the baby on the mattress produced transient pressure changes in the system, which were registered by means of a Statham PM5