epithelial tissues, dissolving pathways toward the epithelial surface (Fig. 2d). Viewed under light microscopy at this stage, the epithelium appears vacuolated; the connective tissue supports of the gill filaments have been largely destroyed and normal filamentary plications have disappeared (Fig. 2, a and b).

Ultimately, multiple pathways are digested through the epithelium to the ctenidial surface, with the result that the activity of the granulocytes reduces the integrity of the ctenidial epithelium suprajacent to the massive granuloma. Eventually the weight of the granuloma overcomes the weakened adjacent tissues and it detaches along with associated ctenidial tissue and is sloughed away and eliminated from the mantle cavity as pseudofeces.

Autotomy is uncommon among the Mollusca, and we know of no other instance whereby a neoplasm is naturally excised from affected tissue. The process described here represents a unique adaptation by a bivalve mollusk to eliminate a hyperplasic granuloma. This appears to be a gerontological phenomenon, because the inner demibranchs of clams with shells shorter than 30 mm are rarely observed to be torn. Thus Morton's (*2*) contention of reabsorption of the necrotic tissue may be correct for small clams. Only when the involvement becomes so large as to endanger normal ctenidial function or when clams become old (or large) is the radical step of tissue autotomy taken.

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3. That Morton (*2*) failed to observe the process described here may be explained in part by differences in the size of individual clams in Hong Kong and North America. *Corbicula fluminea* generally does not exceed 30 mm (shell length) in Plover Cove, Hong Kong; it frequently grows to 50 mm or more in many parts of the present North American distribution. Thus, autotomy of large granulomas may be gerontologically induced. Asian specimens may not normally live to the age or attain the body size at which massive granulomas form.
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Purified Reduced Nicotinamide Adenine Dinucleotide: Responses to Lactate Dehydrogenase Isozymes from Three Cell Sources

Abstract. *Lactate dehydrogenase (LDH, E.C. 1.1.1.27) isozymes from three single-cell sources reacted differently with reduced nicotinamide adenine dinucleotide (NADH) purified to published chromatographic and spectrophotometric specifications and free of inhibitors of LDH, when compared with a commercial preparation of NADH. The activity of LDH-1, purified from rabbit erythrocytes, increased the most with inhibitor-free NADH; the next most stimulated were the LDH isozymes from a control hepatocyte line; but hardly responsive at all were the same isozymes from chemically transformed cells. Thus isozyme composition alone did not account for the range of responses to purified NADH. The commercial preparation of NADH used in these studies contains the Strandjörd-Clayson inhibitors, the most potent group identified in NADH preparations relative to LDH activity. The results suggest that specific molecular differences in individual isozymes contribute to the differential response to the Strandjörd-Clayson inhibitors.*

Although reduced nicotinamide adenine dinucleotide (NADH) is required for catalytic activity by over 200 dehydrogenases of plant and animal origin (*1*), it is recognized that commercial preparations of the reductant contain variable amounts of contaminants (*2–4*) known to inhibit at least six dehydrogenases (*5*), as well as individual isozymes of LDH (*6*, *7*). Until now, investigators have not had the opportunity to study dehydrogenases with NADH purified to chromatographic and spectrophotometric specifications and free of inhibitors. Availability of such a preparation (*8*) encouraged the evaluation of steady-state activities compared with a commercial preparation of NADH.

Table 1. The activity of LDH preparations with NADH(A) and NADH(B).

LDH activity (IU/ml-min)*		Percentage difference (B)/(A) × 100
NADH(A)	NADH(B)	
LDH-1, erythrocytes		
203 ± 1.00	236 ± 2.00	16.26
189 ± 1.00	224 ± 0.00	18.52
Mean		17.39
Control hepatocytes		
282 ± 0.00	304 ± 6.00	7.80
300 ± 0.00	334 ± 6.00	11.33
225 ± 3.00	240 ± 0.00	6.67
242 ± 0.00	262 ± 0.00	8.26
Mean		8.52
Chemically transformed hepatocytes		
225 ± 3.00	229 ± 1.00	1.78
208 ± 2.00	214 ± 2.00	2.89
205 ± 1.00	208 ± 0.00	1.46
270 ± 2.00	278 ± 8.00	2.96
244 ± 0.00	256 ± 2.00	4.92
255 ± 3.00	257 ± 1.00	0.78
Mean		2.47

* Values represent the mean and standard deviation.

Table 2. Chromatographic analysis of NADH-(A) and NADH(B). The results are given as percentages of the total area of the chromatographic peaks at 254 nm (see Fig. 1).

Component	NADH(A)	NADH(B)
NADH	93.6	99.08
NAD^+	2.8	0.53
AMP	0.1	0.16
ADP-ribose	0.5	0.12
ADP	0.8	0.11
Nicotinamide	0.5	0
NMN	0.6	0
Peak 2 (Fig. 1)	0.4	0
Peak 1 (Fig. 1)	0.1	0

For these studies, we selected three examples of LDH isozymes isolated from single-cell mammalian sources in order to eliminate isozyme contributions from different cell types present in intact organs. The enzymes are LDH-1, purified from rabbit erythrocytes (*9*), and two preparations from cell lines of rat hepatic origin, one a control line, TRL 12(13) (*10*), and one, NMU-3, transformed in vitro with nitrosomethylurea (*11*). Although the cells of hepatic origin cannot be distinguished by light microscopy, the NMU-3 line produces carcinomas in vivo. Both these lines are characterized by LDH-4 and -5, permitting comparison of the response to purified NADH with the same isozymes of related cell origin.

The activity of the LDH isozymes was determined by the method of Schwartz and Bodansky (*12*) with two preparations of NADH: NADH(A), Boehringer Mannheim, grade I, purchased through regular supply channels and stored over desiccant at 2°C, and NADH(B), purified by the same firm to meet chromatographic and spectrophotometric specifications for inhibitor-free material (*8*), sealed under nitrogen and stored at −20°C. Both samples were protected from light.

The results of these analyses are given in Table 1. The coefficient of variation of the enzyme assays was of the order of 0.7 percent, implying high reproducibility of these results (*13*). The activity of

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Table 3. Spectrophotometric analysis of NADH(A) and NADH(B).

Spectrophotometric properties	NADH(A)	NADH(B)
Ratio absorption (260/340 nm) (theoretical, 2.265)	2.415	2.303
Ratio absorption (260/290 nm) (theoretical, 10.35)	9.83	10.00
$E_{260\ nm}$ ($\times 10^3$) (theoretical, based on $Na_2NADH \cdot 2H_2O$, 14.4)		14.45
$E_{340\ nm}$ ($\times 10^3$) (theoretical, 6.32)		6.27

LDH-1 was stimulated more than 17 percent when assayed with NADH(B). Similar comparison of LDH extracted from the control hepatocytes, TRL 12(13), revealed an increase in activity of 8.5 percent, but only 2.5 percent increase was observed with extracts from the NMU-transformed cells. The results demonstrate a greater sensitivity of LDH-1 to inhibitors in NADH(A). These results parallel those for the response of LDH-1 from cardiac tissue compared with liver isozymes reported by Gerhardt *et al.* (*7*). The results with the two hepatocyte lines are of specific interest. Although the isozyme types were unchanged, there appeared to be an alteration in the catalytic behavior of LDH with chemical transformation of the cells leading to decreased sensitivity to inhibitors in NADH(A). This is in agreement with previous molecular and kinetic results from this laboratory (*14*). The present results demonstrate that isozyme composition alone is not the only factor determining the response to inhibitor-free NADH, and provide additional evidence that this enzyme undergoes molecular changes during transformation of the cells.

Analytical differences between the two preparations of NADH, shown in Fig. 1 and Tables 2 and 3, clarify the basis for the inhibition with NADH(A). Of particular importance in Fig. 1 are the two early-eluting components, peaks 1 and 2, absorbing light at 340 nm. These occur in positions similar to the groups of LDH inhibitors identified previously (*2*) in NADH samples exposed to moisture. The Strandjörd-Clayson inhibitors (*2*) are recognized as the most potent LDH inhibitors associated with NADH preparations; however, chemical identification of this group is incomplete. Figure 1 also reveals several components absorbing at 254 nm. These have been identified as weak inhibitors (*4*). No evidence of the inhibitor 1,6-dihydronicotinamide adenine dinucleotide (*3*), absorbing at 340 nm, is observed in this chromatogram although 2.8 percent NAD^+ is present. Under the experimental conditions, this amount would have been insufficient to form the inhibitory pyruvate-LDH-NAD^+ adducts described by Everse and N. O. Kaplan (*15*). Tables 2 and 3 compare the composition of the two preparations of NADH: NADH(A) is 93.4 percent pure, whereas NADH(B) contains less than 1 percent impurities, including NAD^+ and adenine nucleotides, and none of the other impurities found in NADH(A) (*7*).

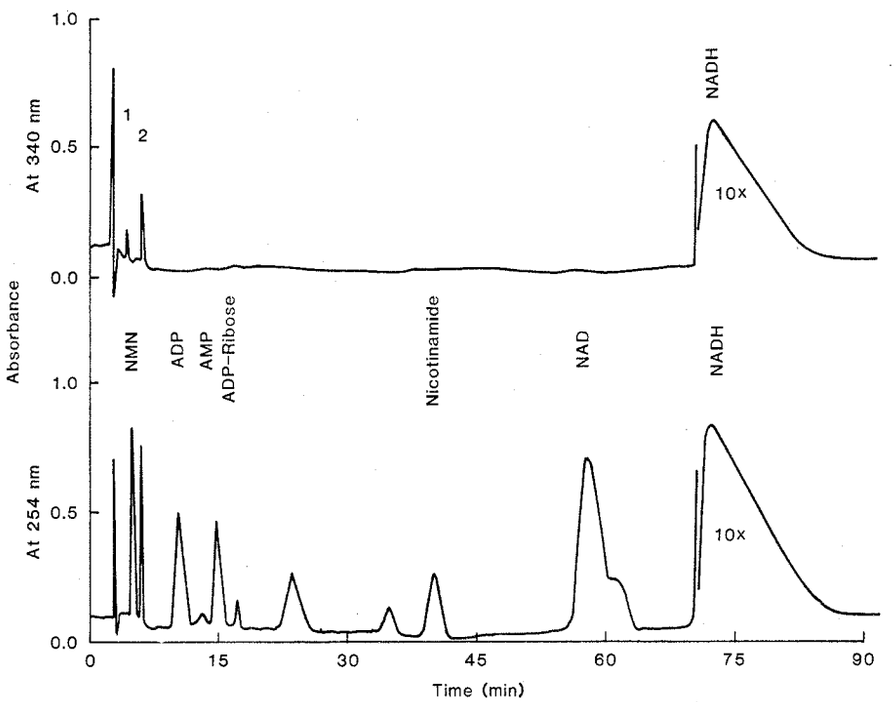

Fig. 1. Reverse-phase high-performance liquid chromatography analysis of the components in NADH(A) (*8*). The NADH (0.5 mmole/liter) in 100 μl of phosphate buffer (0.03 mole/liter), *p*H 7.0, was separated on an octadecyl silane column equilibrated with buffer. The absorbance scale, set at 0.02 Å for 254-nm detection and at 0.01 Å for 340-nm detection, is attenuated as indicated by the values at the breaks in the chromatograms.

The results with the three isozyme preparations indicate that LDH-1 from rabbit erythrocytes is more sensitive to the Strandjörd-Clayson inhibitors than either hepatocyte preparation, although the last two enzymes are not equally sensitive. The need for inhibitor-free NADH for enzyme studies is evident from the range of results with these three LDH preparations as well as from results of earlier studies with LDH isozymes and other dehydrogenases (*2*, *5*–*7*). Furthermore, different preparations of NADH-requiring dehydrogenases (*1*) may react individually to specific inhibitory components. Identification of all of the Strandjörd-Clayson inhibitors is not complete, so the basis for the variations in the sensitivity of the three LDH isozymes to this group of inhibitors cannot be determined. However, the range of responses observed in steady-state analysis suggests the possibility of identifying catalytic differences in these as well as other dehydrogenases by sensitivity to specific inhibitors in NADH preparations (*16*).

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Excitation of *Limulus* Photoreceptors by Vanadate and by a Hydrolysis-Resistant Analog of Guanosine Triphosphate

Abstract. *Discrete voltage fluctuations that occur spontaneously or in response to dim lights can be recorded from the ventral photoreceptors of* Limulus. *The injection of vanadate or the hydrolysis-resistant analog of guanosine triphosphate, GTP-γ-S, into ventral photoreceptors induces the production of discrete waves in the dark. The chemically induced discrete waves are similar to those induced by light. Ventral photoreceptors may contain a guanyl nucleotide binding protein whose activation by vanadate or GTP-γ-S induces the discrete waves.*

Discrete fluctuations of transmembrane potential that occur spontaneously or in response to dim lights have been recorded from various invertebrate photoreceptors (*1*). The discrete voltage fluctuations induced by light appear to result from the absorption of single photons (*2*) and hence are often referred to as quantum bumps or more simply bumps. Shot noise analysis of the response to steady light of the ventral photoreceptors of *Limulus* suggests that the light response results from a summation of these bumps (*3*). Thus, the cellular process responsible for the production of bumps appears to be fundamental to the phototransduction process.

A series of chemical reactions may intervene between absorption of light and the subsequent response (bumps). We sought to manipulate these chemical reactions by exposing the ventral photoreceptors of *Limulus* to pharmacologic agents. When we discovered that exposure of ventral photoreceptors to fluoride ions (F^-) induced bumps in the absence of light (*4*), we suspected that fluoride activated the biochemical pathway that was normally affected by light. However, fluoride affects many biochemical reactions, and we could not define the particular pathway being activated in the photoreceptor. To identify the biochemical steps involved in phototransduction, we looked for other specific agents that might produce bumps. We now report that vanadate (VO_3^-) and guanosine-5′-*O*-(3-thiotriphosphate) (GTP-γ-S), a hydrolysis-resistant analog of guanosine-5′-triphosphate (GTP), induce bumps in the absence of light. All experiments were carried out on the ventral photoreceptors of *Limulus* (*5*).

Figure 1 shows that injection of GTP-γ-S (*5*) induces bumps within the photoreceptor in the absence of light. The recording in Fig. 1A was taken before injection and that in Fig. 1C after the injection; both were taken in the dark. For comparison, the response of the cell (before injection) to a steady dim light is shown in Fig. 1B. The bumps induced by the injection of GTP-γ-S have a similar time course and, on the average, a smaller amplitude than those induced by light. Exposure of cells to fluoride (*4*) also induced bumps of similar time course and smaller amplitude than those induced by light. The effects of GTP-γ-S last for a long time. For the cell in Fig. 1, no sign of recovery was seen for more than 3.5 hours after injection.

Vanadate induces bumps either when added to the artificial seawater (ASW) that bathed the preparation or when injected intracellularly by iontophoresis (*5*). The bumps in Fig. 2 were induced by VO_3^- injection. Injection of vanadate (Fig. 2B) caused a dramatic rise in the rate of occurrence of bumps. The cell recovered only partially from the effects of the VO_3^- injection (Fig. 2, C and D). We found it experimentally easier to control the effects of VO_3^- by adding it to the ASW that bathed the receptor. When added to the ASW at concentrations as

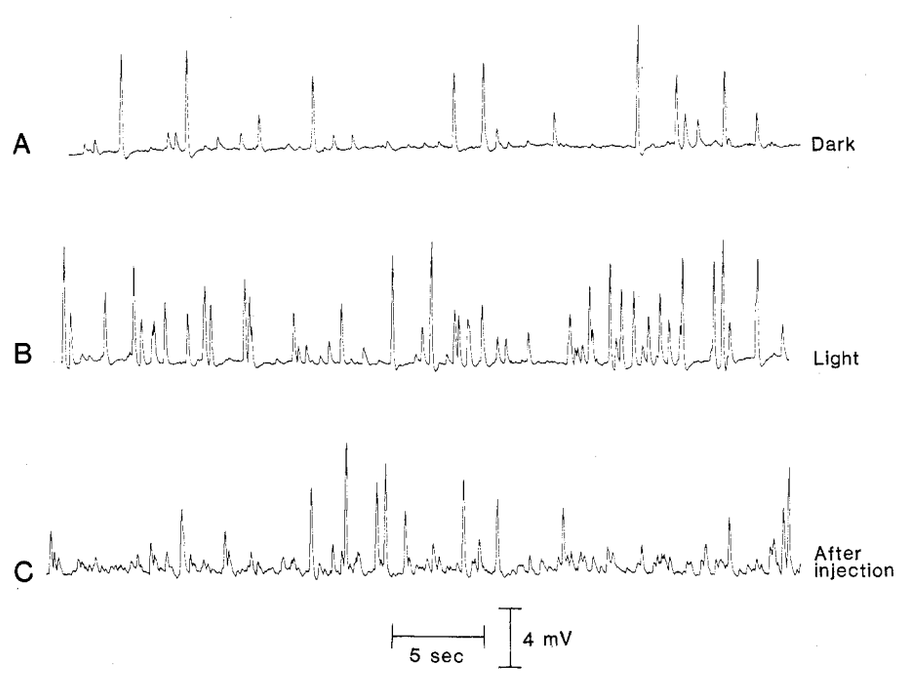

Fig. 1. Discrete waves (bumps) induced by GTP-γ-S. Intracellular recordings from a photoreceptor (A and B) before and (C) after injection of GTP-γ-S into the photoreceptor. The GTP-γ-S was injected into the cell iontophoretically by a 1-nA, 5-second hyperpolarizing current pulse; the pulse was repeated once every 10 seconds for 15 minutes. For purposes of comparison, (B) shows bumps elicited by a steady white light whose intensity was attenuated 8 log units below the maximum intensity available from the light source.

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