mechanical response elicited by the different substrates, the data clearly demonstrates that mechanical performance is affected by the available exogenous substrate in the primate papillary muscle. Not only is τ affected, but the basic parameters ($\dot{\tau}_{max}$ and TPT) which determine the developed tension are also sensitive to the available substrate. Although the method(s) by which substrates modulate mechanical performance remains unclear, the data presented here along with those of other studies[7] demonstrate that a functional dependence exists between mechanical performance and the available substrate.

A striking feature of the data presented in figure 1,B is the negative slope of the butyrate curves (τ vs v and $\dot{\tau}_{max}$ vs v). This occurred for all experimental muscles. Since butyrate was the 2nd or 3rd substrate presented, the negative slope cannot be ascribed to either premature testing during the initial equilibration period or excessive muscle fatigue at the end of the experiment. Instead it would appear that the cardiac reserve in the experimental animals is compromised when presented with a short chain fatty acid as the substrate.

The mammalian myocardium preferentially metabolizes long chain ($\geq$C16) fatty acids[2]. Since butyrate is a short chain fatty acid (C4), the results presented here may not be applicable to the in-vivo heart. However, both long and short chain fatty acids are catabolized via β oxidation and the TCA cycle with only the long chain carnitine transport system being different. This system may[16,17] or may not[18] be directly responsible for the dysfunction during ischemia. Since butyrate does not require this labile transport system and is catabolized via the same pathway as the long chain fatty acids, the data presented here should be applicable to the more physiological in situ situation. These data indicate that the chronic ingestion of an athrogenic diet fundamentally changes the relation between available substrate and mechanical performance.

2 points should be made about the data shown in figure 2. First, unlike most excised mammalian papillary muscle, the treppe continues well beyond the tenth contraction. The cause of the treppe response in the mammalian myocardium is not well understood but may be related to mobilization of sarcolemmal calcium[19,20]. Thus, the protracted treppe may indicate that rhesus monkey papillary muscles mobilize sarcolemmal calcium differently from most other mammals. The 2nd point is that in addition to its slight negative inotropic effect, β-adrenergic blockade by propranolol extends the treppe response even longer. This suggests that there is at least some adrenergic involvement in the protracted treppe.

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Is tubulin involved in the electrically-induced mechanical activity of the isolated rat sciatic nerve?[1]

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Summary. The electrically-induced mechanical activity of isolated segments of rat sciatic nerves remains unaffected following incubation with 10^{-4} M colchicine, vinblastine or melatonin. Vinblastine depressed tubulin levels in incubated nerves. These results suggest that microtubules are not involved in nerve mechanical activity in vitro.

In previous studies we documented a distinct electrically-induced mechanical activity in isolated segments of guinea-pig sciatric nerve[2]. Several reports have indicated the existence in nervous tissue of a relatively high concentration of tubulin, a microtubule protein, which accounts for 11-40% of the total soluble protein[3]. Since various types of intracellular movement, such as axoplasmic transport, have been attributed in part to the microtubules[4,5], it was decided to explore the possiblity of their role in the mechanical activity of isolated and electrically-stimulated nerve preparations.

Methods. Adult Wistar rats (250 g) were killed by decapitation and the distal part of the principal trunk of the sciatic nerve was dissected out. Segments 2 cm long were removed and manipulated as described earlier[2]. Nerve preparations were suspended in Krebs-Ringer bicarbonate solution (KRB) containing 11.0 mM glucose. The medium was bubbled with a mixture of 95% O_2^- 5% CO_2 at a constant pH (7.4) and temperature (37 °C).

Electrical stimuli (square waves of 40 V, 3 msec duration and frequency of 20 Hz) were applied at 10 sec intervals. These values were selected following the previous testing of

different intensities and frequencies of stimulation.[2]. The following parameters were recorded: 1) magnitude of mechanical activity, 2) latency of response and 3) duration of tension. The mechanical amplitude developed by the tissue was expressed in mg and named the isometric developed tension (IDT). Latency between stimuli and responses was measured in sec and the duration of mechanical waves in min.

2 different types of experiments were performed: a) In the 1st series, nerve preparations were allowed to equilibrate in the tissue bath for a 60-min period at 37 °C. Forthwith, they were stimulated as previously stated (trains of impulses delivered every 6 min). An average of the 1st 3 responses was taken as the initial control, after which 3 new identical impulses (at 6, 12, 18 min) were delivered (post-control stimulations), and their responses recorded and expressed as the percent of change compared with controls. The action of melatonin (Sigma Chemical Co.) 10^{-4} M was tested by adding the drug to the tissue bath after the 3 initial control stimulations and percent changes in responses were analyzed. b) Another set of preparations was incubated for 2 hours in KRB kept at room temperature (20–22 °C), mounted for stimulation and the average mechanical response to 5 trains of impulse recorded. Immediately 4 experimental groups were explored: A) untreated controls (incubated in KRB alone); B) KRB with colchicine (Sigma Chemical Co.) at 10^{-4} M; C) KRB with vinblastine sulphate (E. Lilly Laboratories) at 10^{-4} M; D) KRB with melatonin (Sigma Chemical Co.) at 10^{-4} M.

Total tubulin was measured following incubation with the different drugs by estimating the colchicine binding activity in nerve homogenates, as described before[6]. Segments of nerves were homogenized in the cold in 0.067 M sodium phosphate buffer, pH 6.8, containing 0.1 M KCl and 0.1 mM GTP and incubated for 90 min with ^{3}H-colchicine (New England Nuclear Co., Boston, Mass. sp. act. 16.05 Ci/mmole) 2.5×10^{-4} M. The reaction was stopped by adding a 0.1% suspension of activated charcoal, and the mixture was centrifuged at 600 g for 15 min. Radioactivity was measured in an aliquot of the supernatant by liquid scintillation spectrometry, and soluble protein concentration in the same fraction was determined by the method described by Lowry et al[7]. Results were expressed as nmoles of tubutin per mg of soluble protein, assuming that at saturation 1 mole of colchicine was bound per mole of tubulin[6]. Levels of significance between control and experimental values were compared employing Student's t test or analysis of variance and Dunnet's test[8]. Differences were considered statistically significant if $p \geq 0.05$.

Results and discussion. Absolute control values of IDT, duration and latency, as well as percent changes in IDT in post-control stimulated preparations, are shown in figure 1. Addition of melatonin to the incubation medium failed to alter the IDT (figure 1) or the duration or latency of evoked responses. Likewise pre-incubation of sciatic nerves with equimolecular amounts of melatonin, colchicine or vinblastine did not change nerve mechanical responses (figure 2). Under similar experimental conditions only vinblastine affected tubulin concentration of incubated sciatic nerves, by depressing it to 70% of control values (figure 3).

The foregoing results indicate that the electrically-induced mechanical activity of the rat sciatic nerve remains unaltered after incubation with pharmacological agents known to disrupt microtubule function. 2 of these agents (i.e. colchicine and vinblastine) are widely used microtubule inhibitors at the doses tested in the present work[9-11], whereas melatonin has been shown to block fast axonal transport (a microtubul-dependent process) in both optic and sciatic nerves[12,13]. Therefore the present experimental findings support the conclusion that the mechanical activity of the rat sciatic nerve is independent of the intact state of the microtubules. This conclusion is further strengthened by the fact that incubation of nerve segments with vinblas-

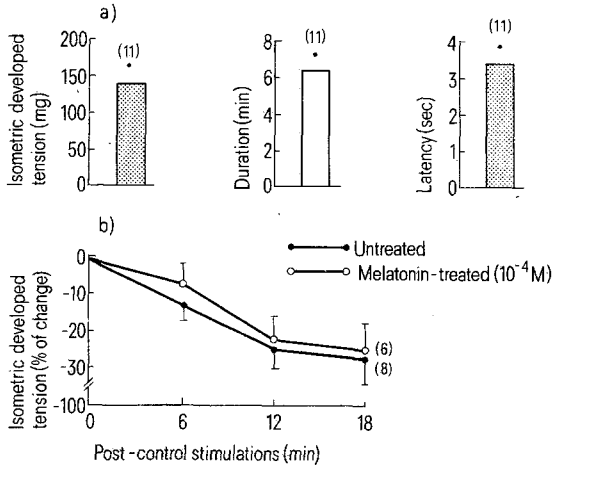

Fig. 1. Isometric developed tension (IDT). Duration and latency of control and post-control stimulations. a) control stimulations. b) % changes of IDT during post-control stimulations in the absence or presence of melatonin. Height of columns and points on curves indicate mean values. Dots on top of columns and vertical bars represent the SEN. For other details see methods.

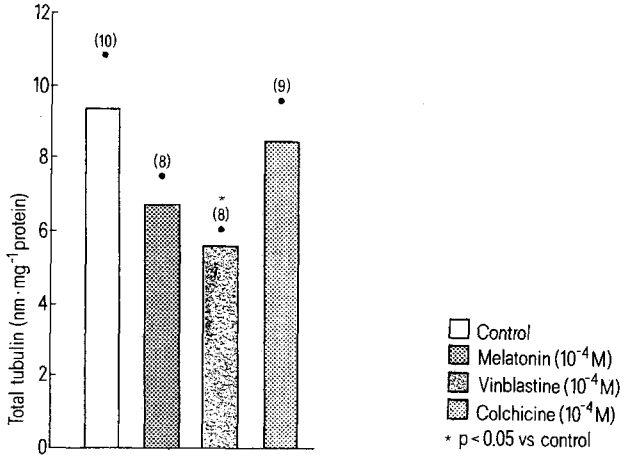

Fig. 2. Effect of melatonin, vinblastine and colchicine on mechanical responses of isolated rat sciatic nerves. Details as for figure 1.

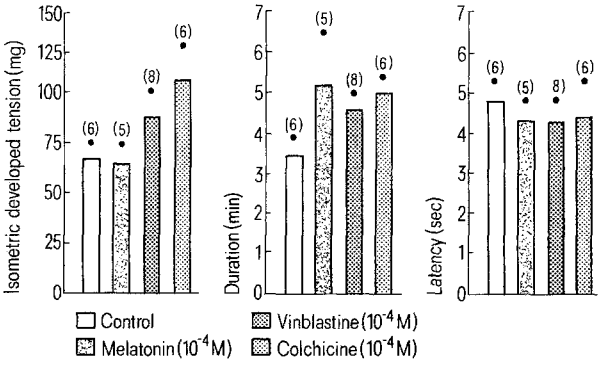

Fig. 3. Effect of melatonin, vinblastine and colchicine on total tubulin of rat sciatic nerves. For details see figure 1.

tine, which resulted in significant depression of the levels of the microtubule-forming protein tubulin, did not affect electrically-evoked mechanical responses in the nerve. Since other contractile proteins (e.g., actin, myosin)[14] are known to be present in neural and perineural cells their possible role in neural mechanical activity deserves further exploration.

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Oxygen equilibrium of *Priapulus* hemerythrin[1]

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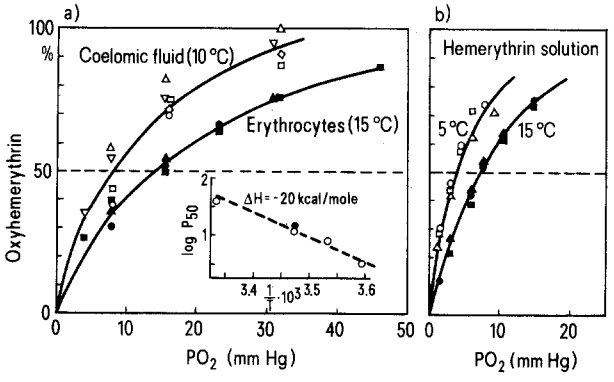
Summary: The hemerythrin-containing coelomic fluid of *Priapulus caudatus* shows a relatively low O_2 affinity (half-saturation O_2 tension $P_{50} = 8$ mm at 10 °C) and a low O_2 capacity (near 1 vol.%). O_2 affinity is independent of pH but shows a large temperature sensitivity. A major role as a continuous O_2 transporter seems to be excluded.

Hemerythrin is the least known of the classes of respiratory oxygen-binding proteins found in animals. It shows restricted phylogenetic distribution, occurring in an annelid *(Magelona)*, most sipunculids, the brachiopod genera *Lingula* and *Glottidia*, and the priapulids *Priapulus* and *Halicryptus*[3]. In contrast to the situation in the former 3 genera, virtually no information is available on the pigment in the priapulids. This paper reports the oxygenation properties of hemerythrin from *Priapulus caudatus*, a large burrowing worm encountered in cold, O_2-rich northern seas[4,5]. It possesses a conspicious, branched anal appendage, whose lumen communicates, via an opening surrounded with circular muscles, with a voluminous coelomic fluid containing hemerythrin-laden erythrocytes; it contracts rhythmically when O_2 availability returns after anoxia[6].

Specimens of *Priapulus caudatus* weighing 3–8 grams were trawled from grey muds at a depth of about 45 m near Kristineberg Marine Biology Laboratory, Fiskebäckskil, Sweden[7]. Coelomic fluid samples were equilibrated with gas mixtures of different O_2 tension (delivered by Wösthoff gas mixing pumps) and the O_2 content of each sample was then measured following a method[8] modified after Tucker[9], where bound O_2 liberated by $K_3Fe(CN)_6$ is recorded using a Radiometer O_2 electrode. Pigment-bound O_2 was obtained after correction for dissolved O_2 (calculated assuming that O_2 solubility in coelomic fluid is the same as in the ambient 35‰ sea water). O_2 saturation at different O_2 tensions was derived expressing the corresponding O_2 content as a percentage of O_2 carrying capacity (defined as O_2 content at atmospheric O_2 tension). O_2 equilibria of erythrocytes washed in sea water and of hemerythrin solutions were measured spectrophotometrically using a modified diffusion chamber technique[10,11].

The coelomic fluid of *Priapulus* showed low values of hematocrit (4–12%) and hemerythrin O_2 carrying capacity (0.4–1.4 vol.%), both parameters increasing with body weight. Oxygenation studies of the coelomic fluid reveal a low O_2 affinity compared to that of most burrowing worms (P_{50} approximates 8 mm at 10 °C), essentially hyperbolic O_2 equilibrium curves (Hill's coefficient, $n \sim 1.2$, reflecting the absence of marked cooperativity between the O_2 binding sites), and the absence of a significant Bohr effect ($\Delta \log P_{50}/\Delta$pH) (figure, a). O_2 affinity, however, decreases drastically with temperature increase (figure, a, inset), reflecting an overall heat of oxygenation (calculated as $2.30R.\Delta \log P_{50}/(\Delta 1/T)$)[12] of about −20 kcal/mole, i.e. almost twice the value generally found with hemoglobins and hemocyanins[12,13]. At the same temperature the washed erythrocytes show an identical O_2 affinity, excluding the possibility of



Oxygen equilibrium of *Priapulus* hemerythrin and its temperature and pH dependence. a) Erythrocytic hemerythrin. Open symbols, whole coelomic fluid; closed symbols, washed erythrocytes suspended in sea water. pH values (varied by admixture of CO_2 to equilibration gases): □, 8.10; ○, 8.05; △, 7.45; ◇, 7.30; ▽, 7.04; ■, 7.62; ●, 7.35; ▲, 7.22. Inset: temperature dependence of oxygen affinity. b) Hemerythrin dissolved in 0.1 M tris buffer of varying pH. pH values: ○, 8.20; △, 7.33; □, 7.05; ●, 8.13; ▲, 7.67; ■, 7.14.