Isometric posttetanic twitch potentiation and staircase is a molecular event strongly associated with in mouse fast muscle. Potentiation of in vitro concentric work is congruent with low-frequency muscle activation the resultant elevation of R-LC phosphate content is congruent with (22). During low-frequency muscle activation the resultant elevation of R-LC phosphate content is congruent with augmented isometric twitch tension (e.g., staircase). Potentiation of both isometric twitch tension (26) in intact muscle and steady-state force in skinned fibers activated at submaximal Ca^{2+} concentrations (18) is thought to result from an R-LC phosphorylation-induced increase in f app, the rate constant that describes the transition of cross bridges from the weak to the strong binding states, while the rate constant for the reverse transition, g app, remains unchanged (24). However, a decrease in g app during steady-state contractions of skinned fibers at submaximal Ca^{2+} activation has also been reported (10), suggesting that if cross bridges left the strong binding states more slowly, force would be enhanced. Thus, at a given submaximal Ca^{2+} activation, a greater fraction of the cycling cross bridges would be in the strong binding states, resulting in an increased extent and rate of isometric force development (26, 27).

Recently, we reported that a 5-Hz 20-s conditioning stimulus that yielded potentiated isometric twitch force coincident with a fivefold increase in R-LC phosphate content also enhanced the work and power of afterloaded contractions by ~22% in mouse extensor digitorum longus muscles (EDL) in vitro. When a potentiated force moves a given submaximal load, upward shifts along the force-displacement and force-velocity relations yield increased work and power (6). However, determination of work and power from force-displacement and force-velocity relations may not reflect muscle performance in vivo (9, 25), because muscles in vivo do not normally move a constant load, nor do they shorten at a constant velocity. For example, muscle displacement in vivo for cats walking on a treadmill demonstrates that muscle length changes (e.g., gastrocnemius) above compared with those below a reference length are not uniform in magnitude, and the periodicity is very much like a sine function (19). Thus a more realistic alternative to assess potentiation of dynamic contractions is the work cycle technique, which can mimic muscle action in vivo in an isolated muscle model. This method has been employed to measure work and power from muscles that generate cyclic movements (e.g., locomotion) in a variety of insects, fish, and mammals (9). If R-LC phosphorylation is to serve a functional purpose in vivo, then work and power should be potentiated during cyclic contractions. To mimic cyclic muscle actions in vitro, but to keep our work cycle model simple, we employed equal-length changes above and below optimal length (L opt) and employed twitch stimulations based on our previous report of potentiated work by mouse EDL during afterloaded contractions (6). The purpose of this study was to determine the work and power responses of...
mouse EDL in vitro to single-twitch stimulations during rhythmic lengthening-shortening sines cycles at 25°C, with the myosin R-LC in a nonphosphorylated and a phosphorylated state.

METHODS

Muscle Preparation

All procedures were approved by the University of Waterloo Animal Care Committee. Two studies were performed using female C57BL/6 mice (21-23 g). In study 1, mouse EDL were subjected to work cycles at three discrete excusions of 0.6, 1.2, and 1.6 mm, which resulted in mean displacements of ~5, 9, and 13% L₀ and at sine frequencies of 3, 5, 7, 10, and 15 Hz to determine the sine frequency within this range for maximum concentric work output. In study 2, the work outputs during the work cycles at the three excursions and a sine frequency of 7 Hz were determined with EDL in a phosphorylated compared with a nonphosphorylated state. In both studies, work was determined at submaximal activation with a single twitch.

Both EDL were obtained from anesthetized mice (pentobarbital sodium, 80 mg/kg body wt ip) and prepared for contractile measures as described previously (26). Briefly, individual EDL were suspended vertically by securing one tendon in a clamp at the base of a jacketed organ bath containing an oxygenated (95% O₂-5% CO₂) Krebs solution (pH 7.4, 25°C) and tying the other tendon with 6-cm of 4-0 suture to the lever arm of a dual-mode servomotor (model 300H, Cambridge Technology, Waterton, MA). Each muscle was equilibrated in oxygenated (95% O₂-5% CO₂) Krebs solution (pH 7.4, 25°C) and a phosphorylated state.

Study 1: Work and Power Across Sine Frequency and Excursion

Before the effect of phosphorylation on work and power output was assessed, the sine frequency for maximal concentric work output at a basal level of R-LC phosphorylation was determined. For each muscle, work sets were obtained at each of the three excursions (~5, 9, and 13% L₀) and for up to three of the five sine frequencies. Muscles rested quietly for 5 min between each set.

Muscles were rhythmically cycled above and below L₀ by a computer-generated sine function driving the arm of the Cambridge servomotor. Data were collected on-line (sample frequency 2,000 Hz) while custom software controlled the frequency and excursion of the sine function and the timing (phase) of the twitch stimulation (Fig. 1) (25). Excursion describes the muscle length change in response to the peak and nadir of the servo arm displacement. Phase describes the relative delay as a percentage of the sine period (e.g., 5, 10, and 15%) between the time of maximum muscle length and the time at which the maximum rate of force development (T of dF/dt max) would occur after a twitch stimulation. At time 0 each sine cycle began by lengthening from L₀. Equation 1 was used to determine the time for the twitch stimulation (T stim) relative to time 0 for a given phase; the example calculates T stim for a phase of 10% at a sine frequency of 5 Hz. With the assumption of a latent period at 25°C of 2-3 ms (6) and a delay from the onset of force development to achieve maximal rate of isometric stress development (dF/dt max) of 2-3 ms (26), a value of 6 ms was employed for T of dF/dt max. T cycle is the sine period (e.g., 200 ms), and T max is the time relative to time 0 at which the muscle achieves its maximum length and then begins to shorten (i.e., 0.25 of T cycle modified equation of Syme and Stevens (25)).

\[
T_{\text{stim}} = \frac{(\text{phase} \times T_{\text{cycle}})}{100} + T_{\text{max}} - T_{\text{dF/dt max}}
\]

64 ms = [(10 × 200 ms)/100] + 50 ms – 6 ms

Each work determination at a given phase required a pair of consecutive sine cycles: the first with no stimulation to account for the passive force response of the muscle to movement of the servo arm and the second with a single-twitch stimulation at a specific phase (Fig. 2). Between each pair of cycles the muscle rested quietly at L₀. Eight different sine cycle pairs were evoked, each for a different phase. Phases were ordered randomly and varied from ~5% (just before the longest length of the muscle) to 30% (approximately L₀) in increments of 5% that yielded predominantly concentric contractions (Fig. 1). A complete work set for a given excursion and sine frequency consisted of eight sine cycle pairs for the eight concentric contractions, for a total collection duration of 8 s (Fig. 2A).

Study 2: Work and Power Potentiation

Work sets were obtained for a given muscle at only one of the three excursions and only at a sine frequency of 7 Hz (see RESULTS). Work was determined under two conditions. In the conditioning stimulus (CS) condition, a group of muscles were subjected to an initial (control) series of work cycles, rested quietly for 5 min at L₀, stimulated while held isometric at L₀ with a 5-Hz 20-s CS, and then subjected to a second (experimental) series of work cycles. On the basis of our previous studies in mouse EDL, R-LC phosphate content at 25°C increases at least four- to fivefold between 10 and 30 s after this CS (6, 15, 26, 27). Therefore, the experimental work series was initiated at 17 s and concluded at 25 s after the CS. In the no-CS condition, control and experimental work series were obtained from a second set of muscles with no intervening CS. In addition, to confirm that the R-LC phosphate content after the control work series and the 5-min period of quiescence in the no-CS condition was near basal levels, a third group of muscles were freeze clamped (liquid nitrogen) at the equivalent of 17 and 60 s after a CS. Modulation of
isometric tension as an index of the presence of an elevated or a basal level of R-LC phosphate content was also determined from twitches obtained 1 min before and 44 s after the final work cycle contraction of the control or the experimental series for the no-CS and CS conditions.

Data Analysis

The degree of R-LC phosphate content in the muscles from the no-CS condition was determined as reported previously (26). Muscle length and diameter, estimated muscle fiber length and total muscle fiber cross-sectional area, maximal active twitch isometric stress (mN·mm$^{-2}$), and $+dF/dt_{max}$ (mN·mm$^{-2}$·s$^{-1}$) were determined as described previously (6). Work was determined from the area of a loop described by a plot of muscle active force (Fig. 3A) vs. muscle displacement (9). The active force was determined by subtracting the raw nonstimulated (i.e., passive) from the raw stimulated force response (Fig. 3A). Active force and displacement were filtered by a dual low-pass Butterworth digital filter (cutoff 100 Hz), and the area within the resultant loop was determined (Fig. 3B; Fig P graphics software, Biosoft, Ferguson, MO). Work was expressed as joules per kilogram of muscle tissue. Work per sine cycle multiplied by the sine frequency yielded mean power (W/kg) (25).

Statistics

Data obtained in study 1 were plotted to provide descriptive profiles of work and power at the various sine frequencies, excursions, and phases employed. Data from study 2 were analyzed by a multivariate analysis of variance; simple effects were examined with a one-way analysis of variance. Tukey's post hoc test was used to determine differences between means. Differences were considered significant for $P < 0.05$. Values are means ± SE.

RESULTS

Study 1: Effect of Phase, Excursion, and Sine Frequency on Work and Power

In this study we wanted to 1) characterize the concentric work and power responses of mouse EDL to single-twitch stimulations elicited at specific points (phase) in a sine function and 2) determine the sine frequency between 3 and 15 Hz that yielded the maximal concentric work output across most of the phases.

Phase. Active work outputs for mouse EDL stimulated by single twitches at sine frequencies that varied from 3 to 15 Hz and mean excursions of 5, 9, and 13% $L_o$ are illustrated in Fig. 4. At 3 and 5 Hz, work rose to a plateau by a phase of 5% and remained relatively constant before decreasing (Fig. 4A). At frequencies of 7, 10, and 15 Hz, over the range of phases employed, work at a phase of −5% was maximal and was enhanced with increasing sine frequency (7 Hz < 10 Hz < 15 Hz; Fig. 4B). After a phase of 0%, work at 7 Hz decreased slightly and then plateaued; work at 10 and
ordered 5% least with respect to the excursion magnitude was 7 Hz the magnitude of work output from greatest to decreased excursion at a phase of frequency (Fig. 4). Output decreased with increasing excursion at a given excursion. However, at phases of 5–20%, active work 0%, the work output was very similar independent of iences. For frequencies of 7, 10, and 15 Hz, at a phase of 30% (Fig. 4). In general, work was maximal for most phases at 7 Hz at each excursion (Fig. 4B); therefore, this frequency was selected to compare work output at all phases with the myosin R-LC in a nonphosphorylated and a phosphorylated state. Power increased linearly with increased sine frequency up to 15 Hz (Fig. 4D).

Study 2: Work With and Without Elevated Myosin R-LC Phosphate Content

In this study we wanted to determine the work output of mouse EDL rhythmically shortened and lengthened at a sine frequency of 7 Hz, performed coincident with a basal level (no-CS condition) or a severalfold increase (CS condition) in myosin R-LC phosphate content.

R-LC phosphate content. We showed previously that R-LC phosphate content 10–30 s after a 5-Hz 20-s CS is 0.60–0.70 mol phosphate/mol R-LC (6, 15, 26, 27). In the present study, R-LC phosphate content determined in muscles freeze clamped at times equivalent to 17 and 60 s after a CS in the no-CS condition were at a basal level of 0.16 ± 0.06 (n = 4) and 0.19 ± 0.03 (SE) mol phosphate/mol R-LC (n = 4), respectively, similar to basal values reported previously (0.08 ± 0.02 to 0.14 ± 0.04 mol phosphate/mol R-LC) (6, 26, 27). These basal levels suggest that R-LC phosphate content after a CS would be increased ~3.7-fold compared with the no-CS condition (e.g., 0.70/0.19 = 3.68).

Isometric twitches. Isometric twitch contractile properties were unchanged by the work cycles (Table 1; see data for control groups for no-CS and CS conditions). Mean stress for isometric twitches obtained 44 s after compared with 1 min before the control or the experimental work cycle series of the no-CS condition and the control work cycle series of the CS condition was not potentiated (Table 1). Consistent with the hypothesis of the present studies, this was interpreted as a low degree of R-LC phosphorylation. In contrast, mean stress and dF/dtmax for isometric twitches obtained 44 s after the completion of the experimental work cycle series in the CS condition were potentiated a mean of 14 and 12%, respectively (Table 1). These potentiated responses were interpreted as evidence of elevated myosin R-LC phosphate content. Half relaxation time was significantly less after compared with before the work cycle series for the CS condition (P < 0.05).

Work and power. The work response pattern at 7 Hz in study 2 was similar to that observed in study 1 with respect to phase and excursion (data not shown). Figure 5 illustrates representative concentric work loops at a phase of 15% for the same muscle in a phosphorylated and a nonphosphorylated state from the CS condition. Work potentiation at each phase is presented in Fig. 6A for the ~9% Lo, excursion. Significant relative potentiation of work output for the CS condition was evident at all phases, whereas only minimal enhancement or depression in work output was observed for the no-CS condition (Fig. 6B). At the ~9% Lo, excursion, relative work was potentiated a mean of 44% for phases of 0–25% (Fig. 6B), at the ~5% Lo, excursion a mean of 25% for phases of 5–30%
DISCUSSION

The purpose of this report was to examine the work and power output of submaximally activated isolated mouse EDL at 25°C during rhythmic lengthening-shortening sine cycles at a low and a high level of R-LC phosphate content. At a basal level of R-LC phosphate content, specific characteristics described the work and power responses of the EDL with respect to sine frequency, phase, and excursion when the EDL was stimulated by single twitches (Fig. 4). Because concentric work output was maximal for most phases and for each excursion at a sine frequency of 7 Hz, this frequency was selected to determine work and power across all phases at an elevated level of R-LC phosphorylation. Coincident with a 3.7-fold increase in R-LC phosphate content, muscle concentric work and mean power were potentiated from 25 to 50% across mean excursions of 5–13% Lo. Peak isometric twitch force and maximal rate of force development were potentiated 14 and 12%, respectively. These data clearly indicate that the utility of a force-potentiating mechanism to muscle function is best revealed under dynamic conditions (e.g., work cycle), instead of from a single

Table 1. Contractile properties for isometric twitches from mouse EDL muscles

<table>
<thead>
<tr>
<th>Variable</th>
<th>No-CS (n = 4)</th>
<th>Experimental</th>
<th>CS (n = 7)</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
<td>Control</td>
<td>Experimental</td>
</tr>
<tr>
<td>Preload, mN·mm⁻²</td>
<td>3.3 ± 0.3</td>
<td>3.3 ± 0.3</td>
<td>3.2 ± 0.2</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>TPF, ms</td>
<td>21</td>
<td>20</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>RT₁/₂, ms</td>
<td>20</td>
<td>15</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Fₐ₀, mN·mm⁻²</td>
<td>35.9 ± 4.0</td>
<td>34.9 ± 4.2</td>
<td>35.9 ± 4.0</td>
<td>34.9 ± 4.2</td>
</tr>
<tr>
<td>dF/dtₘₐₓ, mN·mm⁻²·s⁻¹</td>
<td>3,773 ± 411</td>
<td>3,683 ± 443</td>
<td>3,818 ± 417</td>
<td>3,668 ± 447</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of muscles. Twitches were obtained 1 min before (pre) and 44 s after (post) control (initial series) and experimental (2nd series) work cycles for conditioning stimulus (5-Hz 20-s CS) and no-CS conditions at 25°C. Work cycles were collected at a sine frequency of 7 Hz and an excursion of 1.2 mm [~9% optimal muscle length (Lo); see METHODS]. Forces are normalized to total fiber cross-sectional area (i.e., stress). Preload, muscle resting stress; TPF, time to peak force (or stress); RT₁/₂, half relaxation time; Fₐ₀, active peak stress; dF/dtₘₐₓ, maximum rate of stress development; EDL, extensor digitorum longus. SE for TPF and RT₁/₂ are <1 ms. *Significantly different from pre value within same control or experimental group (P < 0.05).
point on the isometric force profile (i.e., the peak). As expected, when no conditioning stimulus (no-CS) was employed and there was no increase in R-LC phosphate content, neither concentric work, isometric force, nor maximal rate of force development was augmented.

Work output coincident with an elevated R-LC phosphate content was determined at a constant cycle frequency of 7 Hz; therefore, the potentiated net work at each of the three excursions after the 5-Hz 20-s CS was due to augmented force. Although possible, it is unlikely that fatigue mechanisms significantly depressed force output. We previously reported minimal metabolic displacement of stimulated EDL from that of rested muscles after this CS, with only a modest depression in ATP and phosphocreatine (22–27%) and a modest increase in lactate (2-fold) (26). Thus, if elements associated with fatigue such as increased Pi were attenuating force output, their effects were easily masked by mechanisms of force potentiation. Two possible mechanisms to account for the potentiated force after the CS are alterations in Ca\textsuperscript{2+} dynamics, such as increased residual cytosolic Ca\textsuperscript{2+} concentration, or changes in the rates that describe the cross-bridge transitions between the non-force-generating and force-generating states.

Half relaxation time was significantly decreased for isometric twitches obtained after the experimental work series in the CS condition (Table 1); therefore, it is unlikely that increased sarcoplasmic reticulum Ca\textsuperscript{2+} release (e.g., due to increased P\textsubscript{i} (28)) or elevated residual cytosolic Ca\textsuperscript{2+} concentration in the period after the CS can account for potentiated force, work, and power (6, 26, 27). Rather, the diminished half relaxation time suggests less activating Ca\textsuperscript{2+} per twitch stimulation. Therefore, although the potential modulatory effects of Ca\textsuperscript{2+} in the period after the CS cannot be completely discounted because Ca\textsuperscript{2+} was not directly measured, the significant decrease in half relaxation time for the potentiated isometric twitches suggests that force potentiation was not due to Ca\textsuperscript{2+} but likely another mechanism or mechanisms. One such possibility is the increased incorporation of phosphate by the R-LC. This possibility is supported by data obtained from mouse EDL that demonstrate the extent of potentiated displacement (and therefore work) during twitch afterloaded contractions (6) and potentiated work and power during cyclic contractions (unpublished observations) are closely correlated to the extent of R-LC phosphate content over a period of 5 min after the CS.

A current hypothesis to account for steady-state force potentiation in skinned fibers and twitch force potentiation in intact muscle suggests that myosin heads covalently bound with negatively charged phosphate become disordered from the negatively charged thick filament backbone and come in closer proximity to actin, thereby facilitating transition of the cross bridges from the weak to the strong binding states (1, 12, 14, 17). In the cross-bridge model of Brenner (3), this transition is described by the apparent rate constant, f_{app}, a second rate constant, g_{app}, describes the reverse
transition (3). Although in this model Ca$^{2+}$ binding to troponin C is considered the primary regulator of force output by altering the kinetics of actin-myosin interaction through modulation of $f_{\text{app}}$ and not $g_{\text{app}}$ (3), increased R-LC phosphate content appears to have similar modulatory effects independent of Ca$^{2+}$. Coincident with an elevated R-LC phosphate content in skinned fibers of rabbit psoas during submaximal Ca$^{2+}$ activations (e.g., pCa = 5.4) at 15°C, $f_{\text{app}}$ was increased, $g_{\text{app}}$ was unchanged, and the rate of force development and the extent of steady-state force (24) were potentiated. The Ca$^{2+}$ regulation of $g_{\text{app}}$, however, may be temperature dependent, because at 21°C a decreased $g_{\text{app}}$ was reported in skinned fibers of rabbit during submaximal steady-state force contractions (10). Thus, if cross bridges left the strong binding states more slowly rather than, or in addition to, entering these states more quickly, force would be enhanced. To clarify this possibility, the combined influence of temperature and R-LC phosphorylation on rate constants and on steady-state force potentiation should be further characterized. Within the context of the above hypothesis, increased incorporation of phosphate by R-LC could reasonably account for augmented isometric twitch force production and $df/dt_{\text{max}}$ (26, 27), as well as augmented peak work and power from force-velocity and force-displacement relations (6), if $f_{\text{app}}$ (and possibly $g_{\text{app}}$) is modulated in intact muscle as it is in skinned fibers during steady-state force contractions. Furthermore, if elevated R-LC phosphate content has a similar modulatory effect during cyclic submaximal contractions of intact muscle, then work output should be increased. Thus, based on the assumption that increased phosphate incorporation by the R-LC is the predominant response of the muscle to the 5-Hz 20-s CS, we propose, on the basis of available literature, that this biochemical event represents a reasonable mechanism by which work and power during cyclic contractions can be potentiated.

Modulation of muscle force by a potentiating mechanism during dynamic contractions could enhance in vivo skeletal muscle function (6). Movement of the body (e.g., locomotion) is the outcome of a carefully sequenced activation of motor units within appropriate skeletal muscles to provide the force and displacement requirements to articulate the limbs. Muscle work and power to effect movement result from contractions that are characterized by force production and length change over a discrete time interval. The twitch contraction, which represents the fundamental response of a motor unit to a single above-threshold stimulus (5), can clearly be potentiated in humans and in small mammals, yielding increases in isometric force (8) and in work and power (6). Force potentiation in isolated mouse EDL and in human quadriceps appears to be limited to a stimulation frequency threshold of <20 Hz where the force profiles are characterized by unfused twitches (7, 26); thus work potentiation is likely also limited to a similar stimulation frequency threshold. This frequency range has been observed with activation of motor units in human muscles. For example, 60% of the peak force generated by a pool of motor units from human toe extensors was achieved at a stimulation frequency of <20 Hz (4). It should be noted that fusion occurred at a lower stimulus frequency [between 5 and 8 Hz (4)] than in the quadriceps (7), suggesting that the potentiation threshold may vary among muscles.

In humans, muscles are composed of a mixed-fiber population (20), in which slow and fast myosin R-LC can be phosphorylated (21), suggesting that force potentiation may not be limited to only fast or mixed fast motor unit populations, as it is for small mammals (8). With the assumption of a stimulation frequency below the potentiation threshold, if a majority of activated motor units during a movement were potentiated, it would be reasonable to expect a collective potentiated work/power output from the muscle. From a muscle function perspective, a pool of potentiated motor units could yield a substantial gain in work/power output for the same level of neural input. Conversely, to maintain a desired submaximal work/power output, motor unit recruitment and/or firing frequency could decrease, possibly mediated by a mechanical or biochemical feedback pathway.

In summary, we have assumed that increased R-LC phosphorylation is the predominant outcome of a 5-Hz 20-s CS. Potentiation of isometric twitch force in intact muscles (11, 13, 15) or steady-state force in single fibers at suboptimal Ca$^{2+}$ concentration (14, 23, 24) is strongly associated with an elevated phosphate incorporation by the R-LC. Recently, we reported an association between R-LC phosphorylation and potentiated displacement, work, and power on the basis of the force-velocity relation (6). In the present study, coincident with an elevated R-LC phosphate content, work and power

<table>
<thead>
<tr>
<th>Phase, %</th>
<th>Excursion = 0.6 mm (n = 6)</th>
<th>Excursion = 1.6 mm (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Work, J/kg</td>
<td>Mean power, W/kg</td>
</tr>
<tr>
<td>0</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>0</td>
<td>1.12 ± 0.14</td>
<td>1.06 ± 0.15*</td>
</tr>
<tr>
<td>15</td>
<td>1.02 ± 0.12</td>
<td>1.40 ± 0.17</td>
</tr>
<tr>
<td>30</td>
<td>0.73 ± 0.11</td>
<td>0.86 ± 0.12</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of muscles. Data are for representative work cycles at a sine frequency of 7 Hz and excursions of 0.6 (−5% L0) and 1.6 mm (−13% L0) collected 5 min before (pre) and 17–25 s after (post) a 5-Hz 20-s conditioning stimulus. *All post work and power values are significantly larger than corresponding pre values (P < 0.05), except phase of 0 for 0.6-mm excursion.
were also potentiated during twitch activations of a rhythmically cycled muscle. Our findings suggest that R-LC phosphorylation could act to modulate isometric and dynamic muscle contractions during submaximal activations.

This study was supported by funds provided by the Natural Sciences and Engineering Research Council of Canada.

Address for reprint requests: R. W. Grange, Dept. of Physiology, The University of Texas Southwest Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75235-9040.

Received 25 September 1996; accepted in final form 17 September 1997.

REFERENCES