Acidosis stimulates $\beta$-endorphin release during exercise

DEREK V. TAYLOR, JAMES G. BOYAJIAN, NORMAN JAMES, DANETTE WOODS, ALEKSANDRA CHICZ-DEMET, ARCHIE F. WILSON, AND CURT A. SANDMAN
Departments of Psychiatry and Medicine, University of California, Irvine 92668, and State Developmental Research Institute, Fairview, Costa Mesa, California 92626

Taylor, Derek V., James G. Boyajian, Norman James, Danette Woods, Aleksandra Chicz-Demet, Archie F. Wilson, and Curt A. Sandman. Acidosis stimulates $\beta$-endorphin release during exercise. J. Appl. Physiol. 77(4): 1913-1918, 1994.—Elevated blood levels of $\beta$-endorphin have been associated with high-intensity exertion, but the stimulus for $\beta$-endorphin release is unknown. Some studies of exercise have associated $\beta$-endorphin release with increased exertion levels, but other evidence suggests that acidosis may stimulate the release of $\beta$-endorphin. This study examines acidosis as a possible stimulus for $\beta$-endorphin release by examining the effects of arterial blood gases, whole blood lactate, and respiratory changes on $\beta$-endorphin levels and by examining the effects of buffering during exercise on these levels. Initially, seven healthy adult males were evaluated during incremental exercise. During incremental exertion, indicators of acidosis correlated with endorphin release: pH ($r = -0.94$), $\text{PCO}_2$ ($r = -0.85$), $\text{HCO}_3^-$ ($r = -0.89$), base excess ($r = -0.94$), and lactate ($r = 0.89$). A multivariate model showed that $\beta$-endorphin levels were predicted best by the change in base excess. A time course analysis showed that $\beta$-endorphin responses peaked postexercise and paralleled blood acid levels. Subsequently, subjects were compared after alkali loading and placebo during constant-intensity exercise at 85% of maximal exertion to determine whether acidosis is necessary for endorphin release. Treatment with a buffer, which effectively maintained pH above 7.40, significantly suppressed endorphin release ($F = 3.07; P < 0.0001$). The results of this study indicate that acidosis rather than any other physiological change associated with high-intensity exertion is the primary stimulus for $\beta$-endorphin release.

endorphins; lactates; bicarbonates; opiates; respiration

PHYSICAL EXERTION leads to increase in the blood levels of opioid peptide $\beta$-endorphin in humans (10, 15, 17), but the physiological trigger is unknown. Exercise at an intensity $>60\%$ of maximum $\text{O}_2$ uptake ($\text{VO}_2\text{max}$; Refs. 10, 17) or at high muscle work load (15) significantly increases $\beta$-endorphin levels; lower-intensity exertion does not increase endorphin levels (10, 27). Although these studies concur that endorphin release occurs during high-intensity exercise, they fail to identify the physiological trigger responsible for stimulating endorphin release.

Physiological changes occurring at higher-intensity exertion may reveal the mediator of the endorphin response. Several studies suggested that $\beta$-endorphin is released when the anaerobic threshold is reached (3, 17, 23). Because the lactate turn point, characterized by production and release of large amounts of lactic acid (29), is a defining feature of the anaerobic threshold, it is possible that increased blood acidity rather than lactate ion is responsible for the release of $\beta$-endorphin. Several exercise studies showed that $\beta$-endorphin release correlates with both increased lactate production and decreased blood pH (3, 9, 16, 23). A remarkably similar result was found in neonates that had experienced hypoxia (28). In these studies, $\beta$-endorphin levels at birth were closely associated with decreased blood pH, suggesting that acidosis may be the primary stimulus for $\beta$-endorphin release.

If pH rather than lactate levels control $\beta$-endorphin release during exertion, then treatment with a blood buffer should attenuate the endorphin response. The present study examined the relationship between indicators of acidosis (i.e., $\text{arterial pH}$, $\text{HCO}_3^-$, base excess, and lactate) and endorphin release during incremental exertion. The influence of acidosis on endorphin release was specifically examined by buffering pH changes during high intensity exertion at a constant work load.

METHODS

Subjects

The study was approved by the Human Subjects Research Committee of the University of California at Irvine (approval no. 90-231), and each subject provided signed consent before participating. Screening of subjects included medical history, resting electrocardiogram, and spirometry test. Subjects were excluded if they had any evidence of renal dysfunction, cardiac abnormality, respiratory disease, or any other physical condition placing them at risk for maximal exercise testing. Seven healthy adult males between 21 and 46 yr of age were tested. The subjects varied in their levels of physical conditioning. All subjects were in good health and physically active, and six had participated in timed running events $>5$ km. The other subject’s activities included jogging regularly, recreational basketball, and skiing. Subject characteristics are displayed in Table 1.

Procedures

Each subject visited the exercise laboratory on four occasions: 1) screening and orientation to running on treadmill, 2) incremental exercise with blood sampling, and 3) and 4) constant intensity exercise with placebo or buffer, respectively. On the first visit, after screening, each subject completed a graded exercise test to exhaustion following the Bruce protocol (4) as orientation to running on the treadmill. $\text{VO}_2$ uptake ($\text{VO}_2$) and electrocardiogram were measured during 3 min of standing preexercise, exercise, and 10 min of recovery (7 min of walking followed by 3 min of standing).

Respiratory, blood gas, and endorphin measurements during incremental exercise. On the second visit, the subjects returned to complete an incremental exercise test (as above) to study the relationship among arterial blood gases, respiration, and $\beta$-endorphin. One hour before the test, a catheter (22-gauge arterial catheter, Arrow, Reading, PA) was placed in radial artery of the right arm. The catheter was kept patent by continuous flushing with normal saline at a rate of 20 ml/h, and the subject rested for 30 min before an initial sample (7.5 ml) was obtained. Additional samples were collected every 3 min corre-
sponding to the stages of exercise, at maximum exertion, and at 2, 5, and 10 min postexercise.

HCO₃⁻ or placebo loading during constant-intensity exertion.
During the third and fourth visits, subjects completed a 20-min constant-intensity exercise requiring 85% of \( \text{VO}_{2\max} \) as determined from the results of the incremental exercise. The order of treatment with buffer or placebo was randomly balanced and double blind. Each experiment began with the placement of an arterial catheter (see above) followed by placement of an intravenous catheter into the right forearm that was also kept patent with normal saline. After an initial arterial sample was collected, the subject ingested within a 10-min period either 0.3 g/kg of sodium bicarbonate dissolved in 500 mL of water with cherry-flavored syrup or a placebo solution of water with cherry-flavored syrup followed by 1 h of rest to allow for absorption. Buffering was maintained with intravenous isotonic sodium bicarbonate, with the amount calculated from (projected base excess at 85% of \( \text{VO}_{2\max} \) in meq/l) \( \times \) (body weight in kilograms) \( \times \) (0.21 kg body wt of extracellular fluid). Base excess for running at 85% of \( \text{VO}_{2\max} \) for each subject was interpolated from the best-fit curve of base excess vs. \( \text{VO}_{2\max} \). For the placebo experiment, an equal volume of normal saline was infused. The rate of administration was adjusted so that the entire volume would be infused during the preexercise and exercise periods.

Exercise commenced with a 3-min warm-up period at 1.7 mph on a 5% grade followed by a 3-min transition phase and then 20 min at a constant work load, which required \( \text{VO}_{2\max} \) of \( \geq 85\% \) of \( \text{VO}_{2\max} \). Blood samples were taken at the end of preexercise at the end of the warm-up period, every 2 min of exercise, and at 2, 5, and 10 min postexercise. For each subject, both buffer loading and placebo experiments were performed at the same time of day with exercise beginning between 9 A.M. and 12 P.M.

**Physiological measurements.** All exercises were performed on a treadmill (Marathon, California Medical, Brea, CA). Ventilatory measurements during exercise were made on a breath-by-breath basis, averaged, and reported for each 15-s interval. The expired air of the subjects was sampled through a large "J" valve (model 2700, Hans Rudolph, Kansas City, MO; dead space 100 ml) and routed via a capillary tube into a mass spectrometer (model 1100, Perkins-Elmer, Pomona, CA) at a rate of 60 ml/min. \( \text{VO}_{2\max} \), \( \text{CO}_{2\text{production}} \) (\( \text{VCO}_{2} \)), respiratory rate (\( \text{RR} \)), and respiratory exchange ratio (\( \text{R} \)) were calculated from minute ventilation (\( \text{Ve} \)) and exhaled gas measurements. The flow signal from a pneumotachograph (model 50 MC, Meriam, Cleveland, OH) connected to the J valve via 3 m of flexible tubing (3.5 cm ID) was integrated to determine expired \( \text{Ve} \). Continuous cardiac monitoring was obtained with a three-lead electrocardiogram (II, aVF, and V5, Brentwood Instruments, Torrance, CA).

**Blood gases.** Blood gases were measured using blood gas analyzers (Corning 178 and Corning CO-oximeter 2500, Norwood, MA) that measured pH, \( \text{PCO}_2 \), and \( \text{PO}_2 \) by separate electrodes and \( \text{O}_2 \) saturation by spectrophotometry. The first machine also provided mathematically derived \( \text{HCO}_3^- \) and base excess measurements.

**Lactate.** Whole blood lactate was analyzed using a lactate analyzer (model YSI 23L, Yellow Springs Instruments, Yellow Springs, OH).

**\( \beta \) Endorphin.** Blood samples were collected in chilled EDTA venipuncture tubes containing 500 KIU/ml of aprotinin (Sigma Chemical, St. Louis, MO). Plasma was separated by centrifugation (2,000 g; 15 min) and stored in polypropylene tubes at \(-70^\circ\text{C}\) until assayed in duplicate. Plasma \( \beta \)-endorphin levels were assayed by a solid-phase two-site immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). The minimum detectable dose of this method is 14 pg/ml (95% confidence limit) with coefficient of variance of 4.1% (intra-assay) and 9.0% (interassay) at the highest concentration measured in the present study. Cross-reactivity with related opiates (and also oxytocin) is \(<0.01\% \) at 5 \( \mu \)g/ml and that with adrenocorticotropic hormone is 0.028% at 900 ng/ml.

**Statistics**
All statistics were performed using BMDP36 statistical software for the IBM personal computer (BMDP Statistical Software, Los Angeles, CA). A trend analysis of incremental exercise data was performed for each subject to assess the effects of the independent variables (IV) (base excess, lactate, pH, \( \text{PCO}_2 \), \( \text{PO}_2 \), \( \text{HCO}_3^- \), \( \text{R} \), \( \text{RR} \), \( \text{Ve} \), \( \text{VO}_{2\max} \), and \( \text{Ve} \)) on the dependent variable (DV) (\( \beta \)-endorphin) and to obtain a line or curve of best fit for each subject. To more accurately compare the correlation coefficients in light of the small sample size, McNemar's \( r \) to \( z \) transformation (19) was used to reduce the heterogeneity of variance among the subjects. Multiple regression, implementing BMDP program 2R, was used to obtain a weighted linear combination of the IVs (base excess, lactate, pH, \( \text{PCO}_2 \), \( \text{PO}_2 \), \( \text{HCO}_3^- \), \( \text{R} \), \( \text{RR} \), \( \text{Ve} \), \( \text{VO}_{2\max} \), and \( \text{Ve} \)) that best predicted the amount of variance accounted for by the DV (\( \beta \)-endorphin). Experiment-wise type I error was controlled at \( \alpha = 0.05 \) using a Bonferroni adjustment for all planned comparisons and post hoc test of correlation coefficients.

A repeated measures multivariate analysis of variance, BMDP 4V, was performed to assess the effects of the IV "treatment" (placebo vs. buffer) on DV (pH, base excess, \( \text{HCO}_3^- \), lactate, and \( \beta \)-endorphin) over 14 treatments (time points during exercise). Analysis of covariance was used to test the influence of the covariates (blood lactate and \( \text{HCO}_3^- \)) on the DV (\( \beta \)-endorphin).

**RESULTS**

**Incremental Exercise**

The subjects demonstrated high aerobic capacity (\( \text{VO}_{2\max} = 59.4 \pm 5.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)) as shown in Table 1. Figure 1 shows time courses for base excess, \( \beta \)-endorphin, and \( \text{VO}_{2\max} \). Because exercise time varied for each subject, the time scale is expressed as percentage of maximal time so that 100% = end of test (15.8 \pm 4.0 min). \( \beta \)-Endorphin levels peaked at 23.0 ± 2.7 min. This peak was immediately preceded by nadirs of base excess (21.3 ± 4.1 min) and pH (21.7 ± 4.05 min). In contrast, the \( \text{VO}_{2\max} \) peak (15.5 ± 3.23 min) coincided with the end of test.

\( \beta \)-Endorphin levels during rest, incremental exercise, and recovery significantly correlated with pH (\( r = -0.94; P < 0.01 \)), \( \text{PCO}_2 \) (\( r = -0.85; P < 0.01 \)), \( \text{HCO}_3^- \) (\( r = -0.88; P < 0.01 \)), base excess (\( r = -0.94; P < 0.01 \)), and lactate (\( r = 0.89; P < 0.01 \)). Effects during exertion only (recovery

---

**TABLE 1. Subject characteristics**

<table>
<thead>
<tr>
<th>Subj No</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>( \text{VO}_{2\text{max}}, \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>180</td>
<td>82.5</td>
<td>58.7</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>178</td>
<td>73.2</td>
<td>54.7</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>176</td>
<td>65.9</td>
<td>66.2</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>175</td>
<td>68.2</td>
<td>63.9</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>182</td>
<td>78.2</td>
<td>53.6</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>179</td>
<td>67.6</td>
<td>54.4</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>180</td>
<td>74.5</td>
<td>63.1</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>26.7 ± 9.0</td>
<td>179 ± 6.1</td>
<td>72.9 ± 6.1</td>
<td>59.4 ± 5.4</td>
</tr>
</tbody>
</table>

\( \text{VO}_{2\text{max}} \), maximum \( \text{O}_2 \) uptake.
ACIDOSIS STIMULATES ENDOPHIN RELEASE

EXERCISE RECOVERY

END OF TEST

FIG. 1. β-Endorphin, arterial base excess, and O₂ uptake (VO₂) of each subject during incremental exercise test are graphed on standard time line. Time of incremental exercise test was standardized by dividing each time point by total exercise time and expressing it as percent. Separate line type is used for each subject. Data for each subject are represented by best-fit curves. Plots for VO₂ are biphasic linear, whereas those for base excess and β-endorphin are fourth-order quadratic.

and rest values excluded) yielded similar values for pH (r = -0.93; P < 0.01), HCO₃⁻ (r = -0.87; P > 0.01), base excess (r = -0.93; P < 0.01), and lactate (r = 0.94; P < 0.01). VE (r = 0.79; P < 0.05) and RR (r = 0.81; P < 0.05) were also significantly correlated with β-endorphin values.

A stepwise multiple regression was used to determine the best predictor of β-endorphin change. Base excess accounted for all the variance due to acidosis eliminating further need for pH, HCO₃⁻, or lactate in the equation β-endorphin = 52.4 - 8.9(base excess) (R² = 0.88). The strong relationship between base excess and β-endorphin is demonstrated in Fig. 2. The only respiratory measure that accounted for further variance was RR, and the final model R² = 0.93 related changes in β-endorphin to changes in base excess and RR: β-endorphin = 83.65 - 9.43(base excess) - 1.20RR.

Constant-Intensity Exertion

The individually selected treadmill speeds and inclinations, on the basis of the second incremental exercise test, allowed subjects to maintain a constant level of exertion, requiring a VO₂ of 50.9 ± 11.0 ml·kg⁻¹·min⁻¹ or 85.6 ± 15.9% of VO₂max throughout constant-intensity testing. VO₂ did not increase significantly with time of exercise nor was there a significant difference in intensity between placebo (50.6 ± 12.7 ml·kg⁻¹·min⁻¹) and buffer (51.3 ± 9.0 ml·kg⁻¹·min⁻¹) conditions.

Buffering treatments significantly elevated pH values throughout the entire test compared with during the placebo condition (F₁,₁₀ = 28; P < 0.0001), as shown in Fig. 3. Mean pH was 7.43 ± 0.04 in the buffer condition and 7.32 ± 0.05 during the placebo condition. In both conditions, pH decreased significantly with time (F₁₀,₁₅₀ = 26; P < 0.001) during constant-intensity exercise. During the placebo condition, pH dropped from 7.42 ± 0.02 to 7.31 ± 0.05. Buffering elevated pH to 7.50 ± 0.01 at the start of exercise and prevented values at the end of exercise (7.41 ± 0.05) from dropping below the normal resting range. Elevation of pH at the start of the test was due to an
ACIDOSIS STIMULATES ENDORPHIN RELEASE

FIG. 4. Means ± SD of base excess at each time point during constant-intensity exercise for buffer and placebo conditions.

FIG. 5. Means ± SD of blood lactate at each time point during constant-intensity exercise for buffer and placebo conditions.

FIG. 6. Means ± SD of β-endorphin at each time point during constant-intensity exercise for buffer and placebo conditions.

FIG. 7. Adjusted mean β-endorphin values with effects of HCO₃⁻ and lactate as covariates.

Almost eightfold increase in the base excess (from 1.3 ± 1.6 mM in the placebo condition to 10.3 ± 2.1 mM in the buffer condition). Similar to changes in pH (Fig. 4), base excess values were higher in the buffer condition than those in the placebo condition ($F_{1,10} = 52; P < 0.001$) and decreased equally with time in both conditions ($F_{13,130} = 43; P < 0.001$). However, buffering did not prevent mean base excess levels from dropping slightly below zero at the end of exercise for some subjects. Base excess values at the end of exercise were $-9.0 ± 3.3$ meq/l during the placebo condition compared with $-1.6 ± 3.6$ meq/l during the buffer condition.

Although lactate values generally increased with time ($F_{13,130} = 51; P < 0.001$), the effect of exercise time on lactate values was clearly greater in the buffer condition than in the placebo condition ($F_{13,130} = 1.8; P < 0.05$), since lactate increased from $1.0 ± 0.3$ to $9.8 ± 3.2$ mM during buffering but increased from $0.8 ± 0.1$ to $6.4 ± 2.1$ mM during the placebo condition (Fig. 5).

β-Endorphin increased linearly with time across both conditions ($F_{13,130} = 9.0; P < 0.0$), and there was a strong tendency for β-endorphin values to be lower across the buffer condition ($F_{1,10} = 3.87; P = 0.08$, Fig. 6). Using blood HCO₃⁻ and lactate as covariants revealed that treatment with buffer depressed the rate of β-endorphin increase compared with treatment with placebo ($F_{13,130} = 3; P < 0.0001$). Figure 7 shows the adjusted means from the analysis of β-endorphin across time when the effects of lactate and blood HCO₃⁻ are controlled.

DISCUSSION

The findings in this study during incremental exercise indicate that acidosis stimulates the release of β-endorphin and that prevention of acidosis by buffering during high-intensity exercise largely prevents the increase in β-endorphin levels. Changes in measurements of acid-base status, including blood lactate concentration, decreased arterial pH, base excess, HCO₃⁻, and CO₂ levels, significantly corresponded with higher levels of β-endorphin. A multivariate model showed that base excess accounted for 88% of the variance and is the most important variable in predicting β-endorphin response. The small amount of variance that RR accounts for may be the result of reciprocal interaction between the opioid system and respiration.
Manipulation of acid-base status during constant-intensity exertion confirmed that acidosis is the primary stimulus affecting the rate of β-endorphin release. During 20 min of exertion at 85% of \( V_{\text{O}_2\text{max}} \), buffering successfully maintained pH above 7.41. Buffering was accompanied by an increase in the rate of blood lactate elevation and, at the same time, by a suppression of the endorphin response. These effects are opposite those expected if lactate ion were responsible for stimulating the increase in β-endorphin. Other research established that during exercise, when lactate levels exceed the buffering capacity of the cell (0.4 meq/l), lactate diffuses out of the cell in its conjugate acid form and is buffered in equimolar quantities by \( \text{HCO}_3^- \) (2). Therefore, the effect of buffering on blood lactate confounds attempts to maintain a constant acid-base status. Our use of both lactate and \( \text{HCO}_3^- \) as covariates statistically corrected for these effects in a manner consistent with modeling done by Beaver et al. (2). It is evident that \( \text{HCO}_3^- \) loading strongly suppresses the endorphin response even though it increases lactic acidosis.

Although during exercise β-endorphin showed a concomitant increase with respiration, the overall pattern of response suggests an acid-stimulated response. Respiratory measurements (\( \text{VE}, \text{VCO}_2, \) and \( \text{RR} \)) correlated with β-endorphin when only exertion was considered. As seen with other studies (9, 11), β-endorphin values peaked during the 10-min recovery period after exercise, paralleling lactic acidosis, whereas \( \text{VCO}_2, \text{VE}, \) and \( \text{RR} \) decreased immediately at the end of exercise. In this study, considering the recovery period in analysis removed correlations with respiratory measurements, suggesting that these measurements increase coincidentally with β-endorphin and are not causally related. The time lag between acidosis and endorphin response (~1.5 min) is more consistent with the known response latency of β-endorphin to other stresses (14). Therefore, acidosis seems to be the primary stimulus for β-endorphin release. The correlation between respiration and β-endorphin during incremental exercise may be due to the effect of acidosis on these two measurements by separate mechanisms. It has been established that acidosis stimulates respiration through action on chemosensitive neurons in the medullary respiratory control center (8, 13). The hypothalamus is a likely site for pH-sensitive neurons controlling endorphin response because it controls pituitary releases of β-endorphin (1). Therefore, acidosis may effect both respiration and endorphin release but at separate sites.

The response of β-endorphin to acidosis may be consistent with indirect evidence that β-endorphin inhibits respiration during exertion (12, 18) and hypoxia (25). More specifically, blocking receptors for β-endorphin has been shown to have a direct effect on the neurons responsible for respiratory control in the medulla (26). The existence of dual mechanisms for acid-stimulated respiratory changes and β-endorphin release would not exclude acidosis from having a specific role during respiratory challenge. In fact, if the role of β-endorphin is to prevent hyperventilation during respiratory challenge, then acidosis would be an ideal stimulus. Acidosis would stimulate respiration and at the same time stimulate β-endorphin as a feedback inhibitor.

The results of this study have implications for perinatology. Perinatal stress is characterized by unusually high neonatal endorphin levels (6, 7) resulting in respiratory suppression that is treatable with opiate blockers (5). Also, there is evidence that exposure to high endorphin levels during perinatal development may contribute to developmental pathological conditions such as autism (22, 24) and sudden infant death syndrome (21). If buffering of the blood decreases the release of β-endorphin in utero, deleterious effects of endorphin release might be mitigated by fetal alkalinization. Fetuses may be more responsive to the effects of buffering because both acidosis (30) and β-endorphin (20) have been shown to more strongly affect respiratory control centers during early development. Further study in animal models would be useful in determining the therapeutic benefits of buffering treatment.

Although this study examines the effects of buffering in a limited number of conditioned males, it strongly suggests that endorphin release during exertion is stimulated primarily by metabolic acidosis. During incremental exertion, β-endorphin release corresponds with increasing acidosis as measured by arterial blood gases and whole blood lactate. Base excess was the best predictor of β-endorphin release. A time course analysis revealed that β-endorphin mirrored the production of acid with the nadir in base excess closely preceding the peak of β-endorphin during recovery. Most importantly, buffering of blood successfully attenuated the rate of β-endorphin release, confirming that release of endorphin during exercise is dependent on pH changes.

The assistance of Scott Iteen, David Crook, Sheila Tai, Paul Nguyen, and Laura Tai is greatly appreciated. Comments from Penny Fidler were valuable in revising the text.

This study was supported in part by National Institute of Child Health and Human Development Grant RO1-HD-28413-01 to C. A. Sandman.

Address for reprint requests: D. V. Taylor, Research Box 5A, 2501 Harbor Blvd., Costa Mesa, CA 92626.

Received 19 November 1993; accepted in final form 6 June 1994.

REFERENCES


