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Work Unit MR005.04-0082.1

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SUMMARY PAGE

THE PROBLEM

Accurate evaluation of physical fitness is complicated by the factor of motivation and the errors in measurement inherent in current tests. Use of a standardized, reproducible exertion in association with the determination of blood biochemical alterations after exercise might provide a more reliable assessment of physical fitness. This study examines the patterns of serum enzyme changes after exertion and assesses the relationship of these enzyme alterations to physical fitness.

FINDINGS

Two separate elevations in serum glutamic oxalacetic transaminase (SGOT) occur after strenuous exertion. The initial peak is transient and correlates highly with alterations in blood lactate, pyruvate and pH. The second peak occurs about 15 to 24 hours after the exercise and it can be abolished by a physical conditioning program. Resting SGOT concentrations are significantly related to performance in physical fitness tests.

RECOMMENDATIONS

The use of serum enzyme concentrations appears to have significant potential as a measure of physical fitness. Further studies are indicated to evaluate these alterations under other exercise conditions, to study changes in concentrations of other serum enzymes after exertion, and to delineate the pathophysiologic mechanisms involved in these changes.

ADMINISTRATIVE INFORMATION

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This restriction will be removed and the report may be released on 15 April 1968.

ABSTRACT

A study of alterations in selected serum enzymes after physical exertion by young males is reported. Mean serum concentrations of glutamic oxalacetic transaminase (SGOT), lactate dehydrogenase and amylase all increase after the performance of vigorous exercise. SGOT concentrations in the abnormal range are often reached both immediately after exertion to near exhaustion and on the day following vigorous performance of calisthenics. These elevations appear to represent two distinct SGOT peaks. Increments in SGOT concentrations found immediately after exercise correlate highly with alterations in blood lactate, blood pyruvate and blood pH, but not with changes in serum potassium. Thus, the initial peak is associated in some unknown manner with anaerobic glycolysis. The second SGOT peak is probably related to the destruction of skeletal muscle.

Low resting SGOT concentrations correlate significantly with better performances in physical fitness tests. Also, the later SGOT rise is abolished by a 10-week period of physical conditioning. Thus, the measurement of serum enzyme concentrations appears to be potentially useful in the assessment of physical fitness.

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BACKGROUND

Concentrations of numerous serum enzymes increase after vigorous physical exertion[1-10] and the magnitude of this elevation is less in the conditioned subject.[3,4,7] It seems reasonable that a program of exercises leading to improved physical fitness will be associated with adaptive changes in the mechanisms which produce the serum enzyme rises after exertion. This study evaluates the changes in concentrations of several serum enzymes after exertion. The effect of a U. S. Marine Corps recruit physical conditioning program upon the magnitude of changes in serum glutamic oxalacetic transaminase (SGOT) is studied. Finally, alterations in SGOT after a standard exercise test are correlated with alterations in serum potassium, blood pH, blood lactate and blood pyruvate, all of which are known to change with exertion.

MATERIAL AND METHODS

Subjects participating in this study were U. S. Marine Corps infantrymen or recruits at least age 18 and under the age of 26. The experiments were conducted at Marine Corps Recruit Depot, Parris Island, South Carolina, and Marine Corps Base, Camp Lejeune, North Carolina. Parris Island is a Marine Corps recruit training depot. All recruits were carefully supervised and represented a reasonably homogeneous population with regard to age, diet, sleep and physical training. Subjects tested at Camp Lejeune were infantrymen who were participating in various aspects of combat training. Each subject tested at Camp Lejeune was given a physical examination and an electrocardiogram before participating in the study. Acute or chronic illness, a history of repeated infractions of military discipline, or an expression of unwillingness to participate were considered adequate reasons for exclusion from the study.

The results of four studies are presented in this paper. Studies 1 and 2 were designed to elucidate the sequence of serum enzyme changes after exercise. Study 3 was conducted to evaluate the effect of a strenuous physical conditioning program on the serum enzyme alterations induced by exertion. Several biochemical parameters were correlated with serum enzyme changes after exertion in study 4.

A summary of the tests conducted in studies 1-3 is presented in Table 1. All men in studies 1 and 2 had previously run 3 miles as part of the Marine Corps Physical Readiness Test and therefore were assumed to be familiar with their most effective pace. There was no specific training program for the men who ran 3 miles other than that which prevailed in their respective units. Study 3 involved recruits at Parris Island where the physical conditioning program was rigorous and contained both scheduled and extra exercise. Blood

Table 1

Summary of the Tests and Exercises Conducted

Study No.	Purpose	No. of Subjects	Exercise Conducted	Time of Serum Sample	Tests
1	To determine the pattern of serum enzyme changes after exercise	18	3-mi run	Control, and 5 min, 1 and 4 hr, 1, 2 and 3 days after exercise	TS-LDH SGOT
2	To determine the pattern of an enzyme located primarily outside of skeletal muscle	12	3-mi run	Control, and 5 min, 4 hr, and 1 and 2 days after exercise	Serum amylase
3a	To elucidate the effects of a 10-wk recruit training program upon the serum enzyme changes 5 min after exercise	46	600-yd run	Control, and 5 min after exercise	SGOT
3b	To elucidate the effects of the ordinary initial 10-wk recruit training program upon serum enzyme changes approximately 15 hr after exercise	45	600-yd run; 2 periods of calisthenics (see table 2)	Control and approximately 15 hr after final calisthenic period	SGOT

samples were obtained during week 1 and week 10 of the ordinary Marine Corps recruit basic training program. Calisthenics performed on the test days are shown in Table 2.

In study 4, 18 infantrymen participated during a 2-week period at the Naval Medical Field Research Laboratory's climatic chamber. Room temperature was maintained at 75°F with 50% relative humidity. All men were asked to refrain from additional physical exercise during this 2-week period, to obtain

Table 2

Parris Island Calisthenic Schedule

Exercise*	No. Performed
Morning Session	
Side straddle hops	30
Bends and thrusts	15
Push-ups	15
Side straddle hops	30
Push-ups	15
Sit-ups	25
Bends and thrusts	10
Push-ups	15
1-mi run	1
Afternoon Session	
Side straddle hops	40
Bends and thrusts	15
Push-ups	15
Sit-ups	30
Side straddle hops	20
Push-ups	15
3/4-mi run	1

*All exercises were separated by 10 sec of running in place.

adequate rest, to eat breakfast daily, and to abstain from smoking or drinking coffee for at least 2 hours prior to walking on the treadmill. Day 1 was devoted to teaching the men to walk on the treadmill. The second experimental day was used as a sham period during which the men were first instrumented with chest electrodes and then walked for $2\frac{1}{2}$ minutes on the treadmill. This was done to minimize the anticipatory cardiac reflex.

Following the practice session and sham period, all men were studied on three separate days. Each man performed the Modified Balke Test[11] until a designated heart rate was attained. The Modified Balke Test is a treadmill test where the subject walks at 4.5 mph beginning on a level surface with a 1% increase in grade each minute. During each session, the subject walked until the designated heart rate was achieved. The levels required on individual occasions were 148, 164, or 180 beats per minute. The order in which the three tests were performed was randomized by the use of multiple 3x3 Latin squares. At least 48 hours elapsed between each exercise period.

Blood samples were drawn at least 1 hour before the Modified Balke Test, and again immediately after finishing the walk. The subject was instructed to apply pressure to his arm with his hand just before the needle was inserted into the vein and to release when the needle had entered. Blood was then drawn for determination of pyruvate, lactate, pH, SGOT and serum potassium. All blood had generally been drawn by 90-120 seconds after finishing the Modified Balke Test. Samples for pyruvate, lactate and pH were processed immediately. All subjects rested in the sitting position for 10 minutes before their control blood sample was drawn and again prior to walking on the treadmill. Heart rates were recorded on a Sanborn 150 four-channel recorder from a three-lead electrode system on the subject's chest.

SGOT, total serum lactate dehydrogenase (TS-LDH), and serum amyllace determinations were performed by standard techniques.[12-14] After the blood samples were allowed to clot for 30 minutes, the serum was separated by centrifugation. Hemolyzed samples were discarded from the study. Serum for GOT determinations was frozen until it was used while LDH serum samples were kept at room temperature. All determinations were performed within 72 hours after the blood was drawn. Amylase determinations were performed immediately. Versatol E,* Enzatrol** and Blood Chemistry Control Serum† were used to maintain quality control in the enzyme procedures.

Test-retest values for SGOT on 20 unknown serum samples from a hospital population showed a correlation of +0.998. The reliability coefficient for TS-LDH test-retest determinations was +0.972.

Blood pH was measured using a Beckman micro blood pH assembly in a constant temperature block at 38°C. Readings were amplified from a Beckman Zeromatic pH meter onto a Leeds-Northrup galvanometer. Two known buffers, pH 7.44 and 6.86, were used to calibrate the two extremes of the galvanometer scale before each reading. Lactic acid assays were performed in triplicate by a colormetric procedure.[15] Blood pyruvic acid and serum potassium were measured by standard techniques.[16,17]

Intercorrelations were performed with the Pearson product-moment correlation coefficient (r).[18] Student's t test was used to measure differences between means.[19] The 0.05 significance level was selected to evaluate statistical significance. Linear regression coefficients were calculated by the method of least squares.[20] Because of a laboratory error during the determination of blood pyruvate, all calculations involving pyruvate results have an n of 15.

RESULTS

Studies 1 and 2. SGOT, TS-LDH and serum amylase concentrations before and at intervals after a 3-mile run are shown in Table 3. All three mean serum enzyme concentrations were increased at 5 minutes after the run. Both SGOT and serum amylase concentrations had returned to control levels by the next sampling time. A significant difference was present for SGOT between the 4-hour and 24-hour samples. Thus it appears that two distinct SGOT peaks are present, one occurring immediately after the exercise and a second occurring

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** Dade Reagents, Inc.

† Hyland Laboratories.

Table 3

Mean Serum Enzyme Concentrations Over the 72-Hour Period After a 3-Mile Run

	Time After Exercise						
	Control	5 min	1 hr	4 hr	24 hr	48 hr	72 hr
SGOT (Karmen units at 25°C)	21.6	48.1*	19.1	18.8**	24.9**	23.1	21.3
S. D.	6.9	15.5	4.7	3.7	13.8	14.5	10.1
S. E. M.	1.6	3.7	1.1	0.9	3.3	3.4	2.4
TS-LDH (Berger-Broida units)	209.1	244.4*†	257.3*	264.2*†	246.8*	217.9	211.0
S. D.	39.4	48.8	44.1	54.5	48.7	44.1	40.1
S. E. M.	9.3	11.5	10.4	12.9	11.8	10.4	9.5
Amylase (Somogyi units)	64.4	74.9*	-	61.1††	66.1	66.9	-
S. D.	21.9	23.5	-	17.9	23.8	25.1	-
S. E. M.	6.4	6.8	-	5.7	6.9	7.3	-

* Significantly different from the corresponding control mean concentration at a "p" value of ≤ 0.05.

** Significant difference between 4 hr and 24 hr.

† Significant difference between 5 min and 4 hr.

†† Only 10 samples; comparable control value = 61.0.

somewhat later. Serum amylase, which is derived primarily from the pancreas and salivary glands, did not participate in this second elevation. In contrast, TS-LDH continued to rise until the 4-hour sample and remained elevated for at least 24 hours.

Study 3. The results of SGOT measurements before and after a 10-week recruit training program are shown in Table 4. All mean SGOT values were lower during week 10 than during week 1. Running 600 yards was associated with significantly increased SGOT concentrations during the immediate recovery period in both test periods. Although the absolute increase was greater during week 1, the magnitude was proportionately similar (+80%, +99%). The SGOT concentration was also elevated at 15 hours after exercise during the first test period. A comparable elevation was not found during the tenth week.

Table 4

Mean SGOT Concentrations Before and After a 10-Week Physical Conditioning Program

	Time	
	Week 1	Week 10
Control	44.2	17.8
S.D.	39.1	5.0
S.E.M.	5.7	0.7
Five min after 600-yd run	79.8*	35.5*
S.D.	61.0	5.8
S.E.M.	8.9	0.9
Approximately 15 hr after calisthenics	77.4*	19.8
S.D.	73.0	5.2
S.E.M.	10.9	0.8

* Significantly different from the corresponding control mean concentration at a "p" value of ≤ 0.05.

Study 4. Standardized treadmill exercise in the form of a Modified Balke Test produced significant changes from control values in SGOT, blood lactate, blood pH and serum potassium at each of the three levels of heart rate (Table 5). Mean blood pyruvate concentrations were significantly different from control values at only the 180 heart rate level. Increments between control values and SGOT, blood lactate and blood pH after exertion increased as the exercise became rigorous.

Intercorrelation of resting values for the biochemical parameters measured revealed a significant relationship between blood pyruvate and blood lactate (Table 6). All other correlation coefficients were not significantly different from zero although the intercorrelations of both resting blood pH and SGOT with resting blood lactate seem high enough to warrant further evaluation.

Intercorrelations were performed on the increments in the biochemical parameters between control values and concentrations after exertion to the 180 heart rate level (Table 7). All the results of the intercorrelations between increments in SGOT, blood pH, blood lactate and blood pyruvate were significant at the 5% level. Those individuals who walked longer generally had higher blood lactate rises as shown by the significant correlation between the Balke Test results and the increment in blood lactate. Changes in the serum potassium did not correlate significantly with changes in the other parameters measured.

The resting SGOT concentration was found to relate significantly to performance in physical fitness tests. During week 1 at Parris Island, significant correlation (-0.295) was found between the resting SGOT values and the results of the Parris Island Strength Test given to the recruits that same week. This test is composed of pull-ups, push-ups, sit-ups, bend and thrusts, and a 300-yard shuttle run. Results of correlations between the control SGOT concentrations and pull-ups (-0.383) and push-ups (-0.306) were also significant. Since the resting SGOT correlated highly with SGOT values at 5 minutes (+0.984)

Table 5

Mean SGOT, Blood Lactate, Blood Pyruvate, Blood pH and Serum Potassium Concentrations at Three Different Exercise Heart Rates

	Heart Rates		
	148	164	180
Modified Balke Test mean (min)	2.9	5.3	8.4
S. D.	1.8	2.5	3.3
S. E. M.	0.4	0.6	0.8
SGOT (Karmen units at 25°C)			
Control	17.8	20.3	20.2
S. D.	4.3	8.1	9.4
S. E. M.	1.0	1.9	2.2
After exercise	19.6*	25.9*	28.4*
S. D.	5.7	10.9	11.3
S. E. M.	1.3	2.6	2.7
Blood lactate (mg %)			
Control	9.42	9.53	9.72
S. D.	3.33	3.00	3.45
S. E. M.	0.79	0.71	0.81
After exercise	15.26*	30.52*	52.31*
S. D.	6.02	13.20	20.86
S. E. M.	1.42	3.11	4.91
Blood pyruvate (mg %)			
Control	1.76	1.94	1.83
S. D.	0.41	0.41	0.39
S. E. M.	0.11	0.11	0.10
After exercise	1.67	1.99	2.44*
S. D.	0.50	0.43	0.37
S. E. M.	0.13	0.11	0.10
Blood pH			
Control	7.401	7.402	7.405
S. D.	0.023	0.026	0.023
S. E. M.	0.005	0.006	0.005
After exercise	7.331*	7.324*	7.283*
S. D.	0.042	0.050	0.077
S. E. M.	0.010	0.012	0.018
Serum potassium (mEq/L)			
Control	3.94	3.88	3.86
S. D.	0.30	0.32	0.29
S. E. M.	0.08	0.08	0.07
After exercise	4.19*	4.07*	4.22*
S. D.	0.32	0.26	0.34
S. E. M.	0.08	0.06	0.08

*Significantly different from the corresponding control mean concentration at a "p" value of ≤ 0.05.

Table 6

Results of Intercorrelation (r) of Resting Blood pH, Blood Pyruvate, Blood Lactate, Serum Potassium and SGOT Concentrations, and Time Elapsed to Reach the 180 Heart Rate Level in the Modified Balke Test

	Balke Test	Blood pH	Blood Pyruvate	Blood Lactate	Serum Potassium
Blood pH	-0.134	-	-	-	-
Blood pyruvate	-0.158	+0.123	-	-	-
Blood lactate	-0.110	-0.424	+0.786*	-	-
Serum potassium	-0.121	+0.086	+0.117	+0.234	-
SGOT	-0.423	-0.178	+0.040	+0.319	-0.071

*Significant at a "p" value of ≤ 0.05.

Table 7

Results of Intercorrelation (r) of Increments in Blood pH, Blood Pyruvate, Blood Lactate, Serum Potassium and SGOT Concentrations and Modified Balke Test Results at the 180 Heart Rate Level

	Balke Test	Blood pH	Blood Pyruvate	Blood Lactate	Serum Potassium
Blood pH	-0.175	-	-	-	-
Blood pyruvate	+0.280	-0.545*	-	-	-
Blood lactate	+0.560*	-0.791*	+0.676*	-	-
Serum potassium	-0.043	-0.137	+0.283	-0.019	-
SGOT	+0.254	-0.567*	+0.664*	+0.761*	-0.069

*Significant at a "p" value of ≤ 0.05.

and 15 hours (+0.956) after exercise, further intercorrelation of these values with the strength test results was felt to be unnecessary.

A high correlation (+0.62) was found between control SGOT values and the time taken to run 3 miles. Neither TS-LDH concentrations nor recovery SGOT values correlated significantly with performance in the 3-mile run although positive correlation coefficients generally were found. Resting SGOT values do not correlate well with performance in shorter runs such as the 300-yard shuttle run (+0.206) or 600-yard run (+0.100). Performance in the Balke Test also was suggestively correlated (-0.423) with the control SGOT in study 4. All of these data taken together suggest that individuals with lower resting SGOT concentrations perform better in physical fitness tests

DISCUSSION

The findings of this study clearly show that elevated SGOT concentrations in the pathologic range are reached both immediately after exertion to cardiovascular exhaustion and on the day following vigorous performance of calisthenics. It appears from these data that these elevations represent two separate enzyme peaks. In the case of the SGOT and serum amylase, the initial peak after a 3-mile run is transient. Either a prolonged elevation or, more likely, a second SGOT peak occurs on the day after strenuous physical exertion. This second elevation appears to be related to both the amount of physical work involved and to the degree of physical fitness. A 600-yard run does not produce a late elevation (unpublished data), while a 3-mile run produces a small rise and performance of strenuous calisthenics leads to marked SGOT elevations in unconditioned subjects on the day following the exercise. The elevation of TS-LDH cannot be separated into two peaks in the data presented.

The presence of two distinct enzyme peaks has also been suggested by divergent results published from previous studies. Cantone and Cerretelli,[2] using serum aldolase, have described findings quite similar to those in this paper. They found an initial transient rise in serum aldolase that was followed by an elevated resting serum aldolase concentration 24 hours later in the two subjects studied. These authors demonstrated continued elevations of the resting serum aldolase levels in five subjects who performed daily exhausting exercise over a 50-day period. This did not prevent the transient elevation although it appeared to be of a smaller magnitude. Gardner et al.[1,4] noted a rapid return of enzyme levels to normal after moderate exercise, whereas Remmers and Kaljot[8] have described persistent elevations during an officer training program.

Physical training abolished the late enzyme elevation in study 3. This represents one of the most important findings of the study since it may allow objective assessment of physical fitness. Reduction in the late serum rise is expected with increased physical fitness since a constant calisthenic regimen then could be performed relatively more easily.

Study 4 examines the relationship between the initial, transient SGOT elevation and several blood biochemical parameters that are known to change after exertion. Increments in SGOT, lactate, pyruvate, and pH are all highly intercorrelated while changes in serum potassium concentrations do not correlate well with these other parameters. Since alterations in lactate, pyruvate and pH are clearly related to anaerobic glycolysis,[21] it seems possible that the early SGOT rise may also be based on changes produced by the anaerobic conditions. Zierler,[22, 23] in <u>in vitro</u> studies, has demonstrated that anoxia, glucose lack and metabolic inhibitors all increase the efflux of aldolase from muscle. An alternative hypothesis is that the enzyme changes and the degree of anaerobic glycolysis are both reflections of the severity of exercise, but not causally related. Since correlation techniques do not define cause and effect, but rather describe closeness of relationships, no conclusive statements in this regard can be made.

Since a 600-yard run would not be expected to be associated with significant fluid loss by sweating, hemoconcentration is an unlikely explanation for the serum enzyme changes immediately after exertion. Moreover, since the SGOT concentrations double, this explanation would require contraction of the extracellular fluid volume by 50%, an unlikely event. Allowing the blood to clot for 30 minutes before separating the serum should not explain differences from control values since all samples were treated in the same manner. However, it is possible that the lower pH of the blood that is drawn after exertion favors increased efflux of SGOT from the red blood cells <u>in vitro</u> while clotting occurs. This certainly does not explain the later serum enzyme changes since blood pH should equal control levels at those times. The possibility that the increases in serum enzyme concentrations immediately after exertion reflect an activation of previously inactive serum enzyme must also be considered. This seems unlikely since numerous serum enzymes rise after exertion, thus requiring that all of these be present in a heretofore undescribed inactive state.

The second enzyme peak may be related to the destruction of skeletal muscle precipitated by vigorous exercise. Cases of myoglobinuria in apparently normal individuals are well documented after extremely strenuous muscular work.[24-26] Nineteen cases occurring after repeated squat jumps have been reported from Parris Island.[27] Moreover, striking serum enzyme elevations are invariably present in post-exertional myoglobinuria. Ono[28] has shown that myoglobinuria occurs in most athletes after vigorous exertion. He has also presented evidence to suggest that a change occurs on repeated exertion to make the skeletal muscle resistant to destruction. This is compatible with my observations of a lessened late enzyme elevation after exertion in conditioned subjects.

Pearson et al.[29] have reported a case of idiopathic paroxysmal myoglobinuria in whom enzyme rises occurred at about 5 hours and lasted until at least 26 hours after mild exercise. The time sequence of the findings of Pearson et al. are compatible with the interpretation that the late enzyme elevation seen in this study represents destruction of skeletal muscle. This conclusion is strengthened by the finding that serum amylase levels do not increase later than 4 hours after exercise. Amylase is derived largely from pancreas and salivary glands.

The use of a biochemical test for the evaluation of physical fitness would be advantageous since it would obviate both the factor of motivation and the errors in measurement inherent in the present tests. Thus far, little predictive value for purposes of physical fitness testing has been obtained from the determination of biochemical parameters. However, significant correlation exists between the results of several fitness tests and resting SGOT values. This suggests that further study of the relationship of serum enzymes to performance may be fruitful, especially when other enzymes and fitness tests are used. Since this study clearly demonstrated that physical conditioning eliminates the late enzyme elevation after a set of exercises that had previously produced marked changes, the use of late enzyme changes may also prove valuable in assessing the degree of physical fitness.

The findings in this paper have definite clinical significance. The late enzyme peak is similar, at least in its time sequences, to the enzyme elevation seen in myocardial infarction. Since this second enzyme alteration has not been adequately emphasized, the statement has been made that serum enzyme elevations associated with exercise should not pose a problem in the differential diagnosis of acute myocardial infarction.[9] This statement now appears to be unacceptable. Myocardial infarction is frequently preceded by vigorous exertion. The use of SGOT measurements may be misleading in the differential diagnosis of chest pain after exertion since elevation may reflect alteration in skeletal muscle rather than myocardium. Further study is warranted to determine the effect of age upon this phenomenon and also to elucidate whether an elevation occurs in enzymes more specific for myocardial damage.

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13. ABSTRACT

A study of alterations in selected serum enzymes after physical exertion by young males is reported. Mean serum concentrations of glutamic oxalacetic transaminase (SGOT), lactate dehydrogenase and amylase all increase after the performance of vigorous exercise. SGOT concentrations in the abnormal range are often reached both immediately after exertion to near exhaustion and on the day following vigorous performance of calisthenics. These elevations appear to represent two distinct SGOT peaks. Increments in SGOT concentrations found immediately after exercise correlate highly with alterations in blood lactate, blood pyruvate and blood pH, but not with changes in serum potassium. Thus, the initial peak is associated in some unknown manner with anaerobic glycolysis. The second SGOT peak is probably related to the destruction of skeletal muscle. (U)

Low resting SGOT concentrations correlate significantly with better performances in physical fitness tests. Also, the later SGOT rise is abolished by a 10-week period of physical conditioning. Thus, the measurement of serum enzyme concentrations appears to be potentially useful in the assessment of physical fitness. (U)

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Physical fitness						
Physical fitness testing						
pH						
Potassium						
Pyruvate						

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