CHAPTER II

MATERIALS AND METHODS

1. Experimental Protocols.

1.1 In this study, healthy male subjects were selected by using the pulmonary function test by spirometer (Warren Collin, U.S.A.), and electrocardiogram test. Subjects who had FEV_1 less than 83% of vital capacity and/or the electrocardiogram showed cardiac complication were excluded from this study.

1.2 One hundred and four healthy Thai male subjects (older than 17 year-old) were obtained from the selection. They were divided into 4 groups in according to age and each groups were again divided into subgroups of aerobic-trained and untrained by interviewing of physical activities (questionnaires in APPENDIX I) Aerobic-trained groups are the groups of subjects who performed aerobic exercise at least 30 minutes in every other days or not less than 3 days a week. The period of aerobic exercise in this group is not less than 12 months and still maintained exercising activity prior to the test (Pollock, Foster, Knapp, Rod and Schmidt, 1987). Untrained groups are the groups of subjects who do not perform regular aerobic exercise in daily life.

1.3 Procedures of Exercise Tests.

1.3.1 Weight and height of subjects were measured.

1.3.2 Measurement of 3 aerobic parameters in 2 exercise tests. The test 1 was set to measure $\dot{V}o_{2max.}$ and anaerobic threshold. From test 1, the values of $\dot{V}o_{2max.}$ were plotted against work load to define the values of work load at 50% $\dot{V}o_{2max.}$ for each subjects. Then, these values of work load were used to set the test 2. Therefore, the values of oxygen uptake kinetics measured in test 2 were the values of oxygen uptake kinetics at 50% $Vo_{2max.}$ for each subjects. The test 2 was performed a week

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after the test 1. Time constant of oxygen uptake kinetics at 50% $Vo_{2max.}$ (τ) of the same subjects were defined.

1.4 Criterias to terminate exercise tests (more details in APPENDIX II).

1.4.1 The attainment of $\dot{V}o_{2max.}$ of 17-40 year-old aerobic-trained, untrained, and more than 40 year-old aerobic-trained subjects.

1.4.2 The heart rate showed 80% of maximal heart rate of more than 40 year-old untrained subjects.

1.4.3 Subjective symptom such a discomfort appearance of subjects.

1.4.4 Electrocardiograph showed abnormal electrocardiogram.

1.4.5 Abnormal blood pressure responded.

1.4.6 Malfunction of any equipments.

2. Materials and methods.

One hundred and four healthy Thai male older than 17 year-old were included in this study. The questionnaires were filled up (APPENDIX I) and analyzed. The subjects were divided into eight groups by aged and by daily exercise activity history.

Before the experimental day, all subjects were advised to have a rest for 6-8 hours before performing the tests (Hill, Heymsfield, McMannus and DiGirolano, 1984; Yoshida, 1986) and omitted heavy diet 3-4 hours prior to the tests (deVries, 1983).

On the experimental day, the subjects were :

2.1 measured for weight and height (Detecto, U.S.A.).

2.2 recorded vital sign and blood pressure (Eudameter, Germany).

2.3 During the exercise tests, heart rate of each subject was monitored for prevention of cardiac hazard by :

2.3.1 Heart rate of subjects who were under 40 year-old was recorded by three electrodes. One electrode was placed on the 5th intercostal space, midclavicular line, another two electrodes were at the manubrium and ground at the lower back of the

subjects. The signals were recorded by wide band AC preamplifier (7P3A, Grass Instrument Inc., U.S.A.) and amplifier (7 DAC, Grass Instrument Inc., U.S.A.).

2.3.2 For subjects who were 40 year-old or older, their ECG were monitored for preventing cardiac hazard by using the Bio. Scope 5211.5211R ECG Monitor (Fukuda M-E Kogyo, Japan) which could show twelve leads on ECG tracing.

The blood pressure of each subjects were also measured in the left arm by a barometric sphygmomanometer (Erkameter, Germany) in every 2 minutes.

2.4 Measurement of Aerobic Parameters from test 1 and 2. Two exercise tests were performed by letting the subjects to sitting on a mechanically braked cycle ergometer (Monark, Sweden). The seats could be adjusted appropriately for each subjects (Astrand and Rodahl, 1986; Rodeheffer, Gerstenblith, Beard, Fleg, Becker and Weisfeld, 1986).

2.4.1 Test 1 : Measurement of $\dot{V}o_{2max.}$ and anaerobic threshold. Measurement of $\dot{V}o_{2max.}$ could get directly from the exercise test. Subjects were advised to rest on cycle ergometer for 10 minutes. The room temperature, humidity, oxygen and carbon dioxide percentage were recorded.

During the experimental period which was showed in figure 7, the subjects were allowed to breath room air through a mouthpiece (a Liter Respirometer mouth piece, Collins) which was connected to low resistance breathing by a three-way valve with a dead space of 90 milliliter. The temperature of ventilation ($T_{V_{E,\ ATPS}}$) was recorded from a thermometer at this point. A pneumotachograph (Fleisch i/a 7320 (2#), Instrumentation Associates Inc., U.S.A.) was joined with the expired side of this three- way valve. Two air outlets of the pneumotachograph were united to two arms of differential pressure transducer (PT5A, Grass Instrument Inc., U.S.A.). Signal from the pressure transducer was amplified with low-level DC preamplifier (7PIE, Grass Instrument Inc., U.S.A.) which was further amplified by drover amplifier (7DAC, Grass Instrument Inc., U.S.A.). The amplified signal was then joined to an integrator

(7P10A, Grass Instrument Inc., U.S.A.). Both output from the low-level DC channel and the integrator output were recorded on chart paper. The first output represented the expired air flow rate, the latter represented the total volume of expired air ($\dot{V}_{E,ATPS}$) of each breaths. When there was no air flowing through the pneumotachograph (at the end of the expiration, throughout the inspiration and up to the end of the inspiration phase) the signal from the integrator was automatically adjusted to zero. Under expiration, the increasing of integrator signal reach its maximum at the end of expiration, and automatically adjusted back to zero again. The beginning of inspiration up to the beginning of the next inspiration was the criteria for "one breath".

The expired air from the pneumotachograph was also connected to a bottle of 13 liters mixing chamber (Figure 8) for further detectable of gas composition. Small lumen sampling lines from oxygen analyzer (OM-11, Beckman Instrument Inc., U.S.A.) and carbon dioxide analyzer (LB-2 Beckman Instruments Inc., U.S.A.) were inserted into an upper hole of the mixing chamber. The gas analyzer outputs were sent to a multi-channel polygraph (7 DAC, Grass Instrument Inc., U.S.A.). So that expired oxygen and carbon dioxide percentage (% $O_{2\ E,S}$ and % $Co_{2\ E,S}$) were recorded on chart paper too.

Thermo'probe (Surface Temperature-Banjo Type 408, U.S.A.) was inserted into the mixing chamber at the same site but not attached to the sampling lines. The probe connected to Tele thermometer (Temp I, Thailand) for recording temperature in the chamber, which was the temperature of expired gas ($T_{\%gas,ATPS}$).

The subjects exercised following audio-visual feedback which rhythm monitored by metronome (Seiko, Japan). The metronome was set at 50 round per minute. The pedaling was started at 300 kilopond-meter and 150 kilopond-meter in younger and older groups, respectively. The resistance was increased by 50 kilopond-meter every minute until exhaustion or until each subject met at least one criteria for termination of exercise test (APPENDIX II).

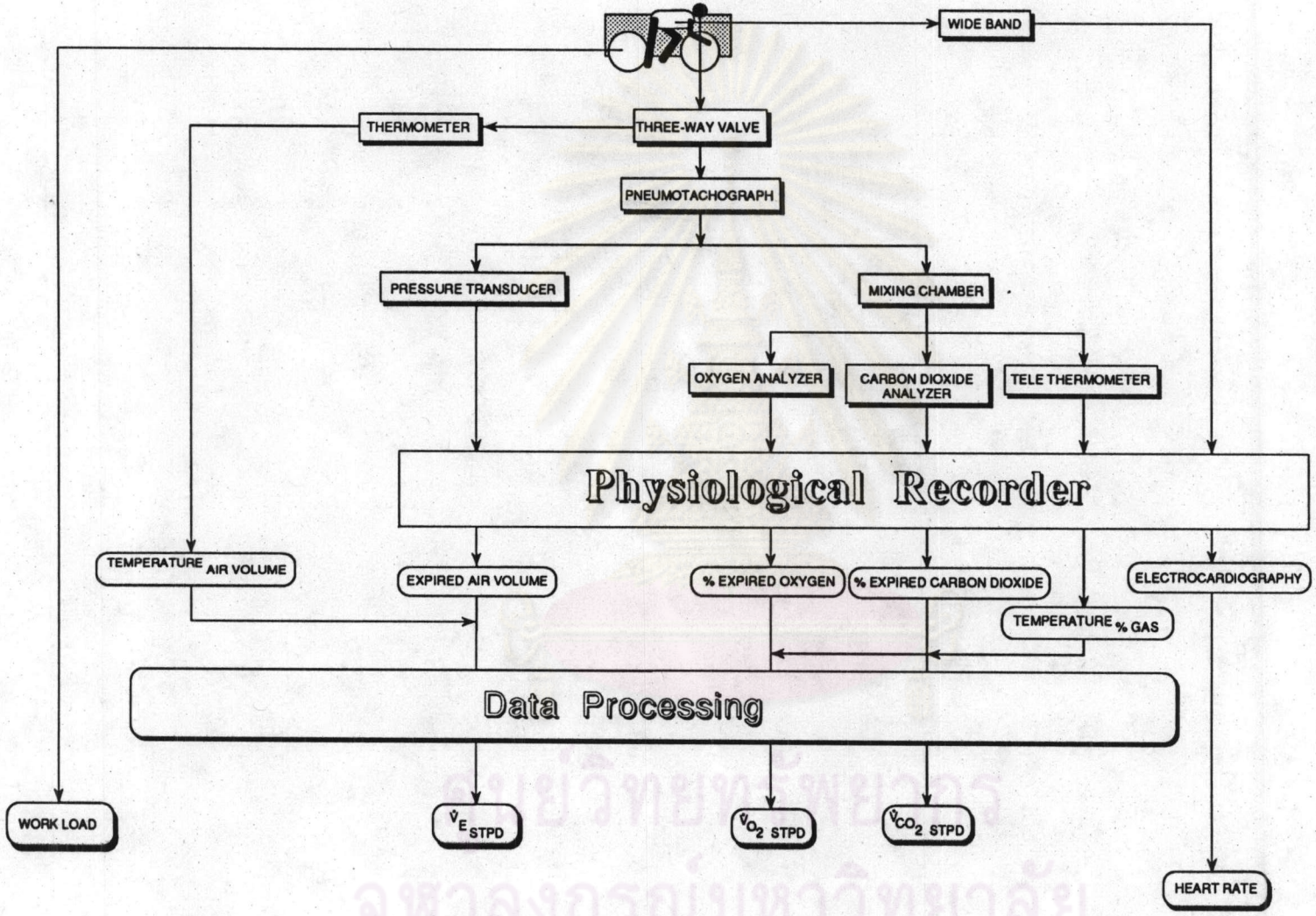

Figure 7. Diagram represented the protocol for expired gas analysis in each exercise test.

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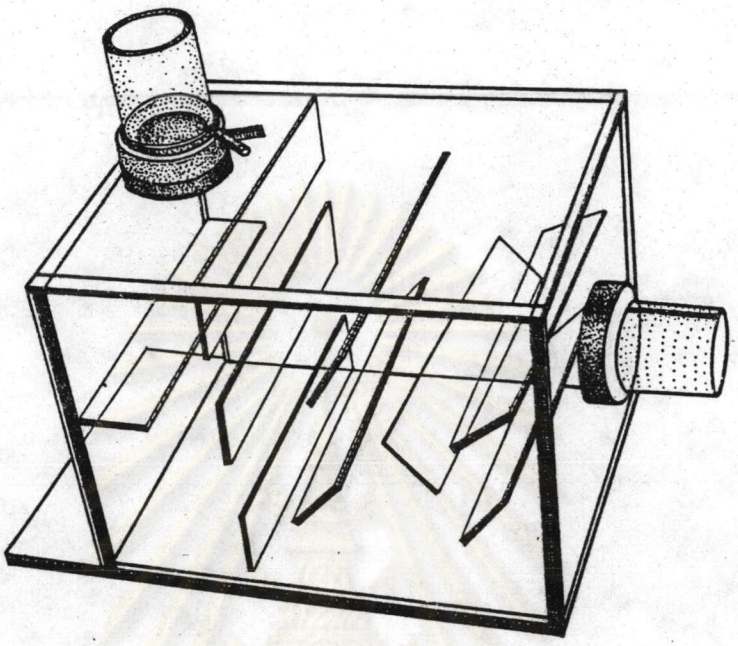

Figure 8. 13 liters mixing chamber for collection of expired gas.

For cooling down purpose, the subjects went on pedaling 0 kilopond-meter (unloaded pedaling) for 8 minutes (Gibbons, Blair, Kohl and Cooper, 1989).

The volume of air ($\dot{V}_{E,ATPS}$), expired oxygen percentage (% $O_{2\ E,S}$) and expired carbon dioxide percentage (% $Co_{2\ E,S}$) were changed to values at STPD and calculated to volume per minute by using equations, showed in APPENDIX III. The highest volume of oxygen in a certain minute were the value of $\dot{V}o_{2max}$.

Anaerobic threshold could be determined from this test 1 too. A special Lotus-based spread sheet was set to plot graphs of : oxygen uptake ($\dot{V}o_2$) against time scale, carbon dioxide output ($\dot{V}co_2$) against time scale, expired air volume ($\dot{V}_E$) against time scale, and $\dot{V}o_2$ against $\dot{V}co_2$ scale. The graphs of $\dot{V}o_2$, $\dot{V}co_2$ and $\dot{V}_E$ against time scale were used to determine the value of anaerobic threshold under two criteria that :- the increasing of $\dot{V}co_2$ and $\dot{V}_E$ was non-proportional to the work load while the increasing of $\dot{V}o_2$ was still linearly to the work load (Figure 4). The graph of $\dot{V}o_2$ against $\dot{V}co_2$

was a V-shape as showed in figure 5. From the method of Beaver and co-workers (1986) called "V-slope" method, the anaerobic threshold could be determined from the point that had a sharp-change of the slope as demonstrated in figure 5.

2.4.2 Test 2 : Measurement of oxygen uptake kinetics. The subjects started pedaling with frequency of 50 round per minute for 4 minutes at 0 kilopond-meter (unloaded pedaling), then the work load was increased in equal increments of the previous work load at 50% $\dot{V}o_{2max}$. The subject exercised at this work load for 6 minutes, and 0 kilopond-meter (unloaded pedaling) for 8 minutes again for cool down period.

Volumes of oxygen uptake which were calculated by special Lotus-based spread sheet for every 15 seconds were then transferred to non-linear regression analysis in another Basic-program (APPENDIX IV) that calculated and presented the volume of oxygen at steady state, rate constant, and time constant (τ) of each subjects in this test 2.

2.5 The setting of Instruments in these tests.

Paper speed of multi-channel polygraph (7 DAC, Grass Instrument Inc., U.S.A.) was kept at 2.5 mm/sec in every test.

The pneumotachograph signal was calibrated by using spirometer (Werren Collin Inc., U.S.A.) as standard volume calibrator. Oxygen and Carbon dioxide analyzers were calibrated by using standard gas containing 12% oxygen and 5% Carbon dioxide balanced with N_2 (Instrumentation Laboratory Inc., U.S.A.). Calibrations were performed everyday before tests.

Sampling flow rate for oxygen and carbon dioxide analyzers were set at 600 ml/min and 500 ml/min, respectively.

3. Statistics.

The statistics used to determine the difference of aerobic parameters in each aged groups was F-test (Analysis of Variance) using the statistic computer program

"Epistat". The physiological differences between aerobic-trained and untrained groups were also tested by F-test. A probability of 0.05 or 0.01 or 0.001 was selected as the criterion for statistical significance.

Pearson correlation of SPSS/PC+ was used to determine the correlation of each aerobic parameter with age in aerobic-trained and untrained groups. A probability of 0.001 was selected as the criterion for statistical significance.

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