CHAPTER III

RESULTS

3.1 Effects of anticoagulants on preservation of erythrocyte 2,3-BPG levels.

Since the erythrocyte 2,3-BPG levels may be affected by anticoagulant and storage, different kinds of anticoagulants and time of storage were studied.

3.1.1 The effects of different anticoagulants on erythrocyte 2,3-BPG levels in fresh blood and blood stored at $4^{O}C$ for 5 days.

To study the effects of different anticoagulants on erythrocytes 2,3-BPG levels in fresh and stored blood, heparin, potassium oxalate and sodium fluoride were used as anticoagulants. The blood sample from each of five normal male Wistar rats was aliquoted into 2 sets of tubes containing heparin (50 units /ml blood), potassium oxalate (2 mg/ml blood) and sodium fluoride (6 mg/ml blood). The first set was immediately determined for erythrocyte 2,3-BPG levels. The second set was stored at $4^{O}C$ for 5 days, mixed at interval, and the levels of 2,3-BPG were determined. Values for 2,3-BPG level were expressed in three different ways: μmole. ml^{-1} packed cells, μmole. g^{-1} Hb and μmole. 10^{6} RBC. The results of 2,3-BPG levels in fresh and stored blood when heparin, potassium oxalate or sodium fluoride was used as anticoagulants were shown in Table 3-1 and Figure 3-1. There was no significant difference in erythrocyte 2,3-BPG levels in all three types of anticoagulated blood sample when immediately determined. However, after 5 days of storage at $4^{O}C$, the erythrocyte 2,3-BPG levels of all anticoagulated blood was decreased, the level was about 48.08%, 32.47% and 4.03% lower than fresh blood as anticoagulating with heparin, sodium fluoride and potassium oxalate, respectively. It is noteworthy that the erythrocyte 2,3-BPG levels were not significantly decreased when the blood was anticoagulated with potassium oxalate and stored at $4^{O}C$ for 5 days.

Similar results were obtained when the levels of erythrocytes 2,3-BPG were expressed as μmole per ml of packed red cells or per g of hemoglobin or per 10^6 RBC (Table 3-1). From these results, the levels of 2,3-BPG in erythrocytes were expressed as μmole per ml packed cells and potassium oxalate was used as an anticoagulant throughout this study.

Table 3-1: Effects of anticoagulants on erythrocyte 2,3-BPG levels between fresh blood and blood stored at 4°C for 5 days.

Type of anticoagulants	2,3-BPG (μmole/ml packed cells)		2,3-BPG (μmole/g Hb)		2,3-BPG ((μmole/10^6 RBC)	
	fresh	stored	Fresh	stored	fresh	Stored
Heparin	4.93 ± 0.77	2.56 ± 0.78[a]	15.10 ± 2.36	7.84 ± 2.39[a]	2.03 ± 0.32	1.05 ± 0.29[a]
Potassium oxalate	5.11 ± 0.83	4.91 ± 0.97	15.67 ± 2.55	15.04 ± 2.98	2.10 ± 0.34	2.02 ± 0.36
Sodium fluoride	5.24 ± 0.63	3.52 ± 1.09[a]	16.05 ± 1.93	10.80 ± 3.35[a]	2.15 ± 0.26	1.45 ± 0.45[a]

The levels of erythrocyte 2,3-BPG were expressed as mean ± SD (n=5)

[a] significantly different from fresh blood, $p<0.05$

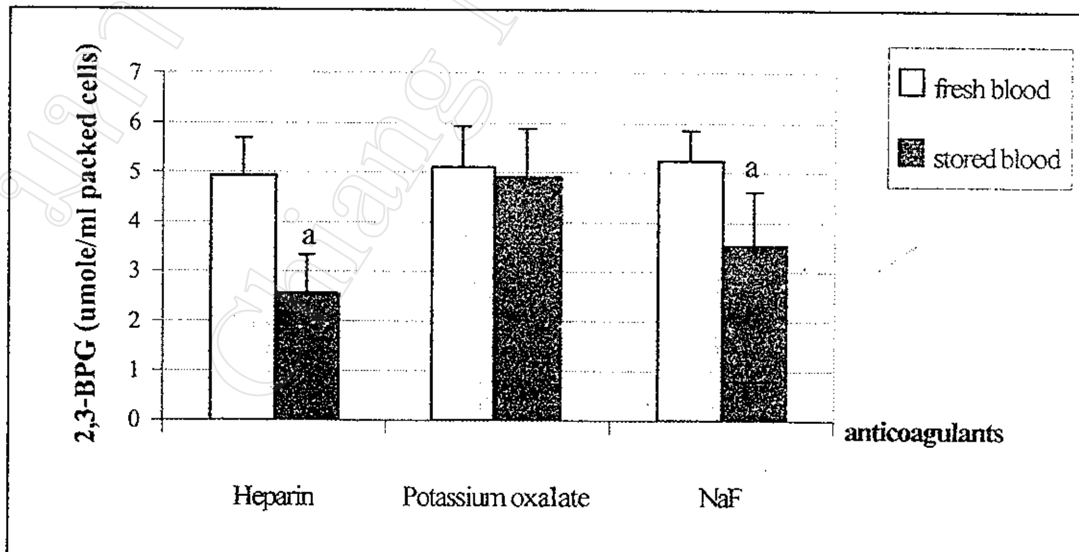

Figure3-1: Effects of anticoagulants on erythrocytes 2,3-BPG levels between fresh blood and blood stored at 4°C for 5 days.

The levels of erythrocyte 2,3-BPG were expressed as mean ± SD (n=5)

[a] significantly different from fresh blood, $p<0.05$

3.1.2 The effects of storage on erythrocyte 2,3-BPG levels when potassium oxalate was used as an anticoagulant.

To further investigate the effectiveness of potassium oxalate on the preservation of erythrocyte 2,3-BPG levels, the blood was anticoagulated with potassium oxalate and stored at 4 ^{o}C for 30 days. The blood sample from each of five normal male Wistar rats was drawn into potassium oxalate (2 mg /ml blood) containing tubes. Then, blood samples were aliquoted for the determination of erythrocyte 2,3-BPG levels at time zero. The remaining was stored at 4 ^{o}C, mixed at interval, and the levels of 2,3-BPG were determined at every five days of storage until the last 30 days of storage. The results of erythrocyte 2,3-BPG levels in the blood that anticoagulated with potassium oxalate and stored at 4 ^{o}C for 30 days were summarized in Table 3-2 and Figure 3-2. There was a gradual decrease in erythrocyte 2,3-BPG levels after storage and the levels were decreasing continuously with time. The erythrocyte 2,3-BPG level was about 4% decreased at the first 5 days of storage, but the difference was not statistically significant when compared to fresh blood ($p>0.05$). For ten days, the storage caused 9.89% depletion and the depletion were 18.95%, 29.27%, 40.40% and 51.15% after 15, 20, 25 and 30 days of storage, respectively. These results suggested that potassium oxalate had a powerful preservation of erythrocyte 2,3-BPG levels for 5 days at 4 ^{o}C. If the erythrocyte 2,3-BPG levels were not immediately determined the potassium oxalate-anticoagulated blood samples should be kept at 4 ^{o}C and the erythrocyte 2,3-BPG levels should be determined within 5 days of storage.

Table 3-2: Effects of storage on erythrocyte 2,3-BPG level when potassium oxalate was used as an anticoagulant and blood stored at 4°C for 30 days.

Time (days)	2,3-BPG (μmole/ml packed cell)
0	4.45 ± 0.18
5	4.27 ± 0.15
10	4.01 ± 0.31^{a}
15	3.61 ± 0.45^{a}
20	3.15 ± 0.45^{a}
25	2.65 ± 0.30^{b}
30	2.17 ± 0.33^{b}

The levels of erythrocyte 2,3-BPG were expressed as mean ± SD (n=5)

[a, b] significantly different from the level of 2,3-BPG in erythrocyte at time zero (fresh blood), [a] $p<0.05$, [b] $p<0.001$

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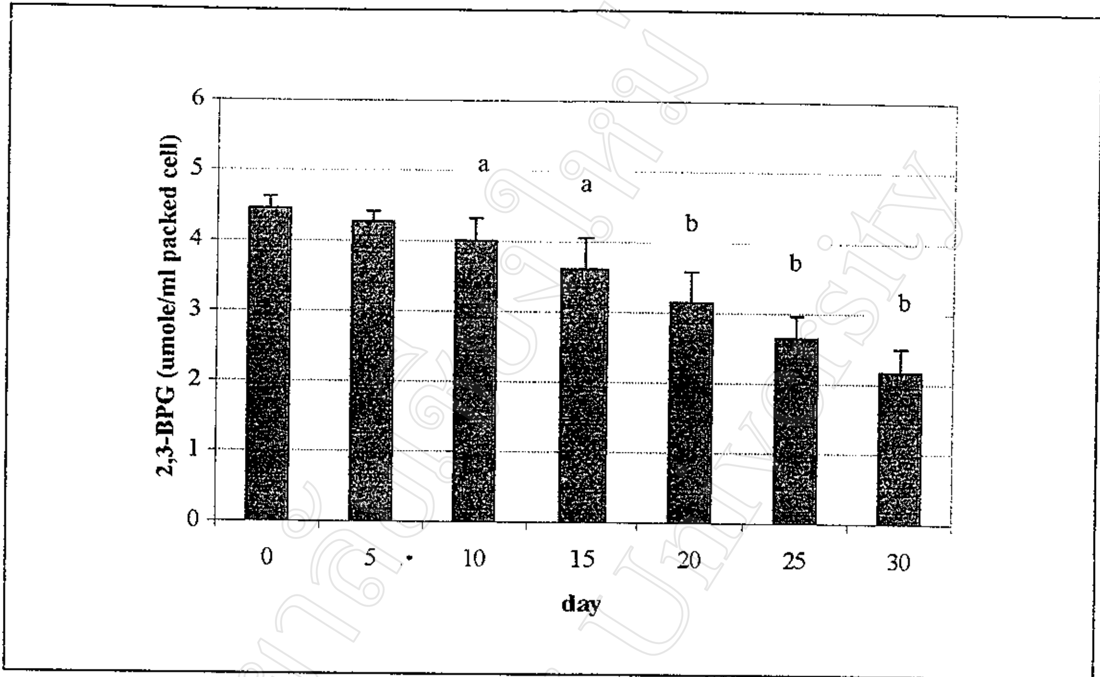

Figure 3-2: Effects of storage on erythrocyte 2,3-BPG level when potassium oxalate was used as an anticoagulant and blood stored at 4°C.

The levels of erythrocyte 2,3-BPG were expressed as mean ± SD (n=5)

[a, b] significantly different from the level of 2,3-BPG in erythrocyte at time zero (fresh blood), [a] $p<0.05$, [b] $p<0.001$

3.2 Effects of exercise programs on carbohydrate and lipid metabolism.

Physical exercise is always accomplished by muscle contraction, which is powered by the high-energy phosphate bonds of ATP. The ATP stored within muscle will meet energy requirements only transiently, for less than 1 minutes. Sustained exercise needs continuously supply of ATP utilization of body energy stores. Fuels available for exercise include fat and carbohydrate, protein utilization requires muscle and parenchymal tissues breakdown. Muscle cells convert fats and carbohydrates to ATP through both aerobic and anaerobic metabolism, but the latter yields less energy, and results in lactic acid accumulation. During exercise, as intensity increases, oxygen uptake must rise in compensation, or the proportion of ATP derived from anaerobic metabolism will increase. Thus, the aerobic metabolism during exercise is maintained by a continued availability of oxygen at the mitochondria. To investigate the adaptation of muscle tissues from anaerobic to aerobic energy metabolism after exercises, the effects of different exercise programs and the alteration of both carbohydrate and lipid metabolism were studied.

3.2.1 The effects of sedentary programs on blood lactate, triglycerides and glucose levels.

To investigate the effects of different exercise programs on .carbohydrate and fat metabolism of the muscle tissues, we determined blood lactate (used as an index of aerobic metabolism), triglycerides and glucose levels after the exercise. At first, the effects of sedentary program on the levels of blood lactate, triglycerides and glucose were studied. Normal male Wistar rats were devided into 3 groups and limited to cage activity for 2, 5 and 8 weeks, respectively. Then, blood samples from each sedentary rats were drawn into potassium oxalate containing tubes. An aliqout of blood was immediately determined for the level of lactate and the remaining blood was centrifuged to obtain plasma for the determination of triglycerides and glucose levels. The results of blood lactate, triglycerides and glucose levels in sedentary control

for 2, 5 and 8 weeks were used as control baseline levels. As shown in Table 3-3, there was not significant change in the levels of lactate, triglyceride and glucose in all three groups of sedentary control. From these results, it was found that the metabolism of carbohydrates and lipids were not changed in sedentary for 2-8 weeks.

Table 3-3: The effects of sedentary programs on blood lactate, triglycerides and glucose levels.

Time (weeks)	Lactate (mM)	Triglycerides (mg%)	Glucose (mg%)
2	0.69 ± 0.33	114.55 ± 17.43	91.33 ± 14.31
5	0.71 ± 0.47	115.49 ± 14.33	92.78 ± 8.99
8	0.69 ± 0.24	114.42 ± 17.02	89.44 ± 9.18

The levels of erythrocyte 2,3-BPG were expressed as mean ± SD (n=6)

3.2.2 The effects of endurance training programs on blood lactate, triglycerides and glucose levels.

To examine that exercise affects changes in the metabolism of carbohydrate and fat, the levels of blood lactate, triglycerides and glucose were determined after training and exercises. Normal male Wistar rats were subjected to run by endurance training programs for 2, 5 and 8 weeks. Then, the blood samples were collected to determine the levels of blood lactate, triglycerides and glucose. The results were shown in Table 3-4. The levels of blood lactate after endurance training for 2, 5 and 8 weeks were significantly increased when compared with sedentary controls ($p<0.001$), This observation suggests an increase of anaerobic energy metabolism during exercises. When compare the levels of blood lactate after endurance training program for 2, 5 and 8 weeks with matched-sedentary control, the level of blood lactate was 65.82% higher within 2 weeks. However, the increase of blood lactate levels were decreased to 59.97% and 50.32 % after 5 and 8 weeks of training, respectively. The level of blood lactate was

decreasing from weekly to week 8. The decrease was significant ($p<0.001$), when compared between week 2 and week 8. In all groups of endurance training program the levels of plasma triglycerides were slightly lower than the sedentary control rats but the difference was not significant. The blood glucose levels were similar among sedentary control and endurance exercise groups. These results suggested that exercise caused higher anaerobic metabolism and training program caused metabolic adaptation from an anaerobic to aerobic.

Table 3-4: The effects of endurance training programs on blood lactate, triglycerides and glucose levels.

Time (weeks)	Lactate (mM)	Triglycerate (mg%)	Glucose (mg%)
2 *	2.02 ± 0.23 [a]	109.12 ± 7.41	91.67 ± 9.60
5	1.78 ± 0.64 [a]	99.77 ± 14.60	90.84 ± 6.21
8	1.39 ± 0.33 [a]	101.97 ± 16.39	88.33 ± 7.64

The levels of erythrocyte 2,3-BPG were expressed as mean (n=6) ± SD, * n=5

[a] significantly different from sedentary groups ($p<0.001$)

3.2.3 The effects of exhaustion training programs on blood lactate, triglycerides and glucose levels.

To confirm that exercise causes an increase in anaerobic energy metabolism in muscle tissues and that training causes muscular adaptation from anaerobic to aerobic metabolism, an intense exercise, exhaustion training program was used in this study. Male Wistar rats were subject to exercise by exhaustion training programs for 2, 5 and 8 weeks. Blood samples were collected after exercise, then, the levels of blood lactate, triglyceride and glucose were determined. These results were summarized in Table 3-5 and showed that the lactate levels after exhaustion training program were significantly increased when compared to the matched-sedentary control ($p<0.001$) and endurance training ($p<0.001$). The exhaustion training caused

87.47% increase of lactate level within 2 weeks and the levels decreased continuously with time to 85.18% and 79.57% after exhaustion training program for 5 and 8 weeks, respectively. The results imply that 2 weeks of exhaust training program caused an increase of lactate levels about 21.65% more than endurance training program.

The triglycerides levels after exhaustion training programs were significantly decreased when compared to the matched-sedentary control ($p<0.001$) and also significantly decreased from endurance training program ($p<0.001$). The reduction of blood triglycerides level was increased continuously with the period of training to 11.86%, 22.80% and 33.29% after exhaustion training program at 2, 5 and 8 weeks, respectively. The level of blood glucose obtained after exhaustion training program were not different from those of sedentary and endurance training program.

These results implied that both endurance and exhaustive exercise caused higher anaerobic energy metabolism at the beginning. However, after longer period of training, rats were adapted to greater extent of aerobic energy metabolism by both endurance and exhaustive exercises, and more triglycerides were used as energy sources. However, different exercise programs according to this study did not cause change in blood glucose level.

Table 3-5 : The effects of exhaustion training program on blood lactate, triglycerides and glucose levels.

Time (weeks)	Lactate (mM)	Triglycerides (mg%)	Glucose (mg%)
2*	$5.51 \pm 0.39^{a,b}$	$91.34 \pm 9.12^{a,b}$	92.22 ± 10.47
5	$4.77 \pm 1.17^{a,b}$	$79.99 \pm 2.94^{a,b}$	92.50 ± 8.87
8	$3.38 \pm 0.47^{a,b}$	$69.13 \pm 8.76^{a,b}$	89.60 ± 8.00

The levels of erythrocyte 2,3-BPG were expressed as mean ± SD (n=6), * n=5

[a] significantly different from sedentary groups ($p<0.001$)

[b] significantly different from endurance training groups ($p<0.001$)

3.2.4 The effects of acute exhaustion and endurance exhaustion programs on blood lactate, triglycerides and glucose levels.

To investigate the effects of acute exhaustion and endurance exhaustion exercises on glucose and triglycerides metabolism, male Wistar rats were subject to limitting in cage activity for 2 weeks and were subject to exercise running until exhaustion on the next day. After exercise, blood samples were collected and the levels of lactate, triglycerides and glucose were determined. The results were presented in Table 3-6. An acute exhaust exercise caused 90.05% increase in lactate levels when compared to matched-sedentary control. The triglycerides levels after acute exhaustion exercise were slightly decreased but not significantly different when compared to sedentary control ($p>0.05$). The levels of glucose were not changed after acute exhaustion exercise. Therefore, it can be concluded that anaerobic metabolism was occurred after acute exhaustion exercise.

To investigate the effects of endurance exhaustion exercises on glucose and triglycerides metabolism, male Wistar rats were subject to run by endurance training program for 2 and 5 weeks and ran until exhaustion on the next day. After exercise, blood samples were collected and the levels of blood lactate, triglycerides and glucose were determined. These results were also summarized in Table 3-6. The increase of the lactate levels were reduced to 88.51% and 85.18% after endurance exhaustion program for 2 and 5 weeks, respectively, but the difference between both groups was not statistically significant ($p>0.05$). The triglycerides levels obtained after 2 weeks of endurance exhaustion exercises were slightly decreased but not significantly different when compared to 5 weeks of endurance exhaustion exercises. The levels of glucose were not changed by endurance exhaustion exercises. The results implied that anaerobic metabolism occurred in endurance exhaustion exercises and training programs caused an adaptation from anaerobic metabolism to more aerobic metabolism.

Table 3-6 : The effects of acute exhaustion and endurance exhaustion programs on blood lactate, triglycerides and glucose levels.

Time (weeks)	Lactate (mM)	Triglycerides (mg%)	Glucose (mg%)
Acute exhaustion 2 weeks	6.93 ± 0.85 [a, b, c]	107.41 ± 9.86	88.11 ± 8.96
Endurance exhaustion 2 weeks	6.00 ± 0.83 [a, b, c]	91.15 ± 16.32	89.80 ± 5.82
Endurance exhaustion 5 weeks*	5.68 ± 0.91 [a, b, c]	90.72 ± 18.97	89.07 ± 11.28

The levels of erythrocyte 2,3-BPG were expressed as mean (n=6) ± SD, * n=5

[a] significantly different from sedentary groups, $p<0.001$

[b] significantly different from endurance training groups, $p<0.001$

[c] significantly different from exhaustion training groups, $p<0.05$

3.3 Effects of exercise programs on erythrocyte 2,3- BPG levels.

From the results shown above, it is obvious that increased anaerobic metabolism occurs during exercise and training causes adaptation of the muscle to more aerobic metabolism. 2,3-BPG, an endogeneous factor in erythrocyte, facilitates the release of oxygen from oxyhemoglobin. Thus, exercise may cause an increase of the erythrocyte 2,3-BPG levels in order for an adequate supply of oxygen to muscle tissues. To investigate the effects of exercise on erythrocyte 2,3-BPG levels, different exercise programs were studied along with glucose and triglycerides metabolism. Male Wistar rats were subject to different exercise programs. Immediately after exercise, blood samples were collected to determine the levels of erythrocyte 2,3-BPG and hematocrit values. The results were summarized in Table 3-7. In sedentary control, the levels of erythrocyte 2,3-BPG were not changed after limiting to cage activity for 2, 5 and 8 weeks. After endurance training exercises, the levels of erythrocyte 2,3-BPG were increased by 15.43% within 2 weeks and were maintained at this level up to 8 weeks later. Exhaustion training exercises caused 19.61% increase of erythrocyte 2,3-BPG levels in 2 weeks and gradually increased to 20.74% and 23.44% after 5 and 8 weeks, respectively. It is noteworthy to note that

8 weeks of exhaustion training program caused an increase of erythrocyte 2,3-BPG levels about 8.00% higher than endurance training exercises. The level of erythrocyte 2,3-BPG obtained from acute exhaustion exercise was increased by 11.33% when compared to matched-sedentary control. In the same ways, endurance exhaustion exercises also caused the increase of the erythrocyte 2,3-BPG levels to 16.03% and 17.44% after 2 and 5 weeks of endurance training, respectively. However, the difference of 2,3-BPG levels between 2 weeks and 5 weeks training were not statistically significant (P>0.05). From these results, it can be implied that exercise causes an increase in the erythrocyte 2,3-BPG levels and difference exercise programs affect the erythrocyte 2,3-BPG metabolism by different magnitudes.

Table 3-7: The effects of exercise programs on erythrocyte 2,3-BPG levels.

Exercise programs	2,3-BPG (μmole/ml packed cell)		
	2 weeks	5 weeks	8 weeks
Sedentary control	5.71 ± 0.33	5.67 ± 0.30	5.62 ± 0.56
Endurance training	6.75 ± 0.08 [1,a]	6.70 ± 0.36 [a]	6.64 ± 0.27 [a]
Exhaustion training	7.10 ± 0.13 [1,a,c]	7.15 ± 0.14 [a,c]	7.33 ± 0.25 [a,c]
Acute exhaustion*	6.44 ± 0.33 [b,d]	ND	ND
Endurance exhaustion**	6.80 ± 0.31 [a,e,f]	6.92 ± 0.23 [a,e,f]	ND

ND not done

The levels of erythrocyte 2,3-BPG are express as mean (n=6) ± SD, [1] n=5

* Rats were subjected to run by acute exhaustion program after limited to cage activity for 2 weeks.

** Rats were subjected to run by acute exhaustion program after endurance training for 2 and 5 weeks.

[a, b] significantly different from sedentary program, [a] $p<0.001$, [b] $p<0.05$

[c] significantly different from endurance training program, [c] $p<0.001$

[d, e] significantly different from exhaustion training program, [d] $p<0.05$, [e] $p<0.001$

[f] significantly different from acute exhaustion program, [f] $p<0.05$

3.4 Factors influencing erythrocyte 2,3-BPG levels.

It is known that the principal role of erythrocyte 2,3-BPG is to facilitate the unloading of oxygen from oxyhemoglobin to the peripheral tissues. During exercise, increased oxygen delivery to muscle tissues is required to enhance aerobic metabolism. Factors that affect changes in erythrocyte 2,3-BPG metabolism and regulates 2,3-BPG levels may occur during exercise. Thus, the effects of a decrease in pH, an increase in blood temperature, pO_2 and Ca^{2+} on 2,3-BPG metabolism in erythrocytes during exercise were studied.

3.4.1 Effects of pH on erythrocyte 2,3-BPG levels.

During exercise, if the delivery of oxygen to the working muscles is inadequate, there is an increase in lactate accumulation. The rise in blood lactate decreases venous blood pH (lactic acidosis). Thus, lactic acidosis may affect the erythrocyte 2,3-BPG metabolism. To investigate the effects of acidic pH on erythrocyte 2,3-BPG levels, the blood was drawn from normal male Wistar rat into potassium oxalate containing tube. The erythrocytes were washed three times with normal saline at 37°C and were resuspended in HBSS pH 6.4, 6.6, 6.8, 7.0 and 7.2 at approximately 30% hematocrit. Each suspension was matched with the erythrocyte suspended in HBSS pH 7.4. All of suspension were incubated at 37 ^{O}C for 15 minutes and the erythrocyte 2,3-BPG levels were immediately determined. The results were shown in Table 3-8. These results were expressed as the % of relative concentration of 2,3-BPG between the incubation at experimental pH and physiological pH (7.40). It was found that incubation of erythrocytes in HBSS pH 6.4 and pH 6.6 caused the reduction of erythrocyte 2,3-BPG levels about 8.0 % and 4.26%, respectively, comparing to the incubation in HBSS pH 7.40. In contrast, when the erythrocytes were incubated with HBSS pH 6.8-7.2, the levels of 2,3-BPG were higher when compared to the incubation at pH 7.4. The erythrocyte 2,3-BPG level was 1.72% increased at pH 6.8 and increased to 2.85% at pH 7.0 and to 6.31% at pH 7.2. At pH higher than 7.2, the level of erythrocyte 2,3-BPG trended to decline.

These results implied that acidic pH affected the change of the erythrocyte 2,3-BPG metabolism. High acidic pH (6.4-6.6) caused reduction of 2,3-BPG levels. The erythrocyte 2,3-BPG levels were increased at mild acidic pH (6.8-7.2) and when compared to the physiological pH 7.4, pH 7.2 caused the highest increase of erythrocyte 2,3-BPG levels.

Table 3-8: Effects of pH on erythrocyte 2,3-BPG levels at 37°C for 15 minutes.

pH	2,3-BPG (μmole/ml packed cells)	% change
7.4 6.4	7.40 ± 0.14 6.81 ± 0.06	-8.0
7.4 6.6	7.37 ± 0.06 7.06 ± 0.23	-4.26
7.4 6.8	6.94 ± 0.10 7.06 ± 0.23	+1.72
7.4 7.0	7.09 ± 0.11 7.29 ± 0.06	+2.85
7.4 7.2	6.96 ± 0.10 7.401 ± 0.09	+6.31

The levels of erythrocyte 2,3-BPG were expressed as mean ± SD (n=5).

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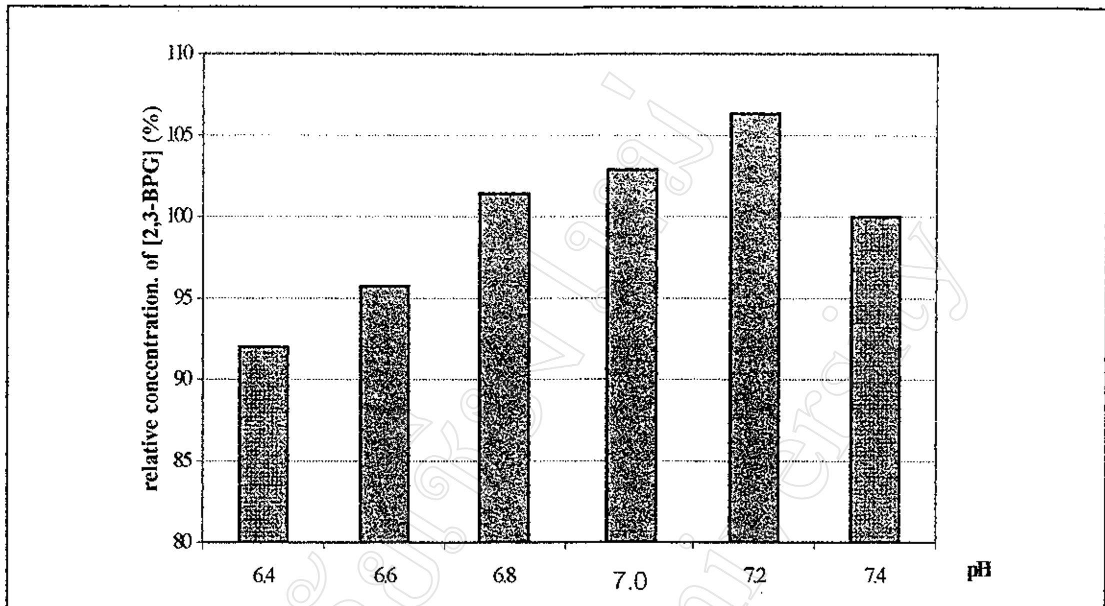

Figure 3-3: The effects of pH on erythrocyte 2,3-BPG level at 37°C for 15 minutes. Values were expressed as % of relative concentration of 2,3-BPG between the incubation at experimental pH and physiological pH.

3.4.2 Effects of temperature on erythrocyte 2,3-BPG levels.

During exercise, an elevation in temperature as a result of increased energy metabolism causes the blood temperature to increase. Thus, elevation in blood temperature may also affect erythrocyte 2,3-BPG metabolism. To investigate the effects of an elevated temperature on erythrocyte 2,3-BPG levels, the blood was drawn from normal male Wistar rat and collected in potassium oxalate containing tube. The washed erythrocytes were resuspend in HBSS pH 7.4 at approximately 30% hematocrit. All of the suspensions were placed in an incubator at different temperature (35°C, 39°C, 41°C, 43°C and 45°C) for 15 minutes, each suspension was matched with the incubation at 37°C and the erythrocyte 2,3-BPG levels were immediately determined. The results were shown in Table 3-9 and, in Figure 3-4, the results expressed as % of relative levels of erythrocyte 2,3-BPG between the incubation at experimental temperature and at 37°C. Incubation in HBSS pH 7.4 at 35°C caused the levels of erythrocyte 2,3-BPG 3.06% lower than that at 37°C. The erythrocyte 2,3-BPG levels were gradually increased as the incubation

temperature was higher than 37°C. The erythrocyte 2,3-BPG levels were 1.36% increased at 39°C and increased to 4.18% and 6.99% after 41°C and 43°C, respectively. However, when the incubation temperature was higher than 43°C the 2,3-BPG level declined. Therefore, it can be concluded from these results that an elevated temperature affected an increase of the erythrocyte 2,3-BPG metabolism. The temperature at 43°C is the optimal temperature that caused the highest increase of erythrocyte 2,3-BPG levels.

Table 3-9: The effects of temperature on erythrocyte 2,3-BPG levels at pH 7.40 for 15 minutes.

Temperature (°C)	2,3-BPG (μmole/ ml packed cells)	% change
35	7.10 ± 0.45	-3.06
37	7.32 ± 0.29	
39	7.25 ± 0.08	+1.36
37	7.16 ± 0.13	
41	7.31 ± 0.13	+4.18
37	7.01 ± 0.08	
43	7.33 ± 0.08	+6.99
37	6.86 ± 0.19	
45	7.10 ± 0.16	+1.90
37	6.96 ± 0.06	

The levels of erythrocyte 2,3-BPG were expressed as mean ± SD (n=5).0

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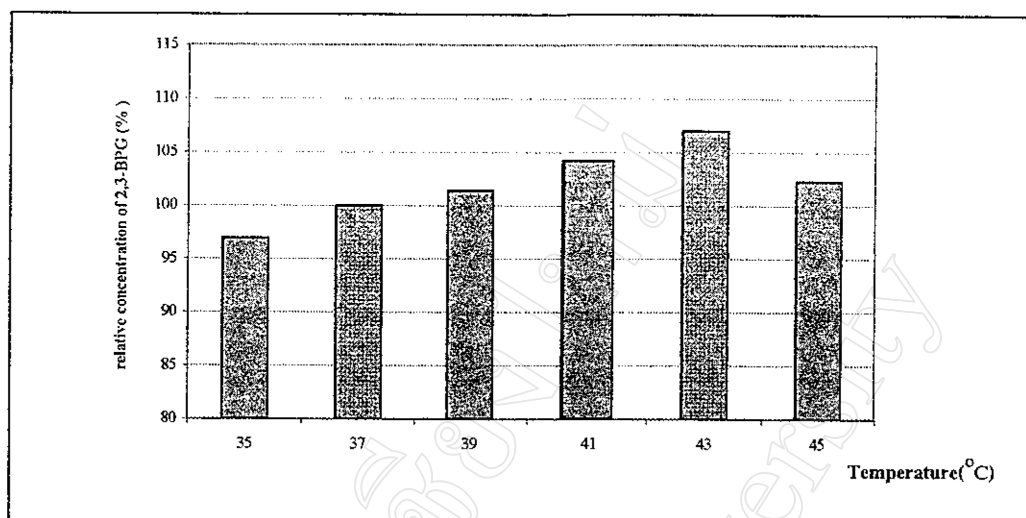

Figure 3-4: The effects of temperature on erythrocyte 2,3-BPG levels at pH 7.40 for 15 minutes.

Values were expressed as % of relative concentration of 2,3-BPG between the incubation at experimental temperature and 37°C.

3.4.3 Effects of A23187 on erythrocyte 2,3-BPG levels.

One of the important properties of an erythrocyte is the ability to transport oxygen from lungs to peripheral tissues. Hemoglobin is responsible for this function and the functional property of hemoglobin is regulating by 2,3-BPG. It is physiologically important by facilitating oxygen release from hemoglobin as erythrocytes are passing through tissue capillaries. During translocation from arterial circulation to tissue capillaries, the erythrocytes can passes through by their flexibility and deformability properties. The deformation of the erythrocyte causes cell membrane to permeabilize to Ca^{2+} ion. During exercise, increased oxygen supply to active muscle tissues is required to maintain aerobic metabolism. Therefore, Ca^{2+} may be another factor that affects the change in erythrocyte 2,3-BPG metabolism during exercise. To investigate the effects of Ca^{2+} on erythrocyte 2,3-BPG levels, Ca^{2+} ionophore (A23187) was used as a tool in this study. Washed erythrocytes obtained from blood of normal male Wistar rats were resuspended in HBSS pH 7.4 at approximately 30% hematocrit. All suspensions were incubated at 37°C for 15 minutes at different concentration of A23187 (2.5, 5.0, 7.5, 10.0, 15.0 nmole/ml blood), matching with the suspensions which were free from A23187 and the erythrocyte 2,3-BPG levels

were immediately determined. The results were summarized in Table 3-10 and Figure 3-6. It was shown that the presence of A23187 in the incubation system caused the increase of erythrocyte 2,3-BPG when compared to the control system. The erythrocyte 2,3-BPG levels were 0.40% increased at 2.5 nmole of A23187 and increased to 1.17%, 3.06% and 11.82% at 5.0, 7.5 and 10 nmole of A23187, respectively. As the concentration of A23187 was higher than 10 nmole/ml blood, the level of erythrocyte 2,3-BPG trended to decline. These results implied that A23187 affected the change in erythrocyte 2,3-BPG metabolism. The presence of A23187 in erythrocyte caused an increase of 2,3-BPG level.

Table 3-10: The effects of A23187 on erythrocyte 2,3-BPG levels at pH 7.40, 37°C for 15 minutes.

[A23187] (nmole/ml blood)	2,3-BPG (μmole/ml packed cells)
0 (control)	6.50 ± 0.11
2.5	6.53 ± 0.20
5.0	6.58 ± 0.14
7.5	6.71 ± 0.20
10.0	7.37 ± 0.31
15.0	7.01 ± 0.17

The levels of erythrocyte 2,3-BPG were expressed as mean ± SD (n=5).

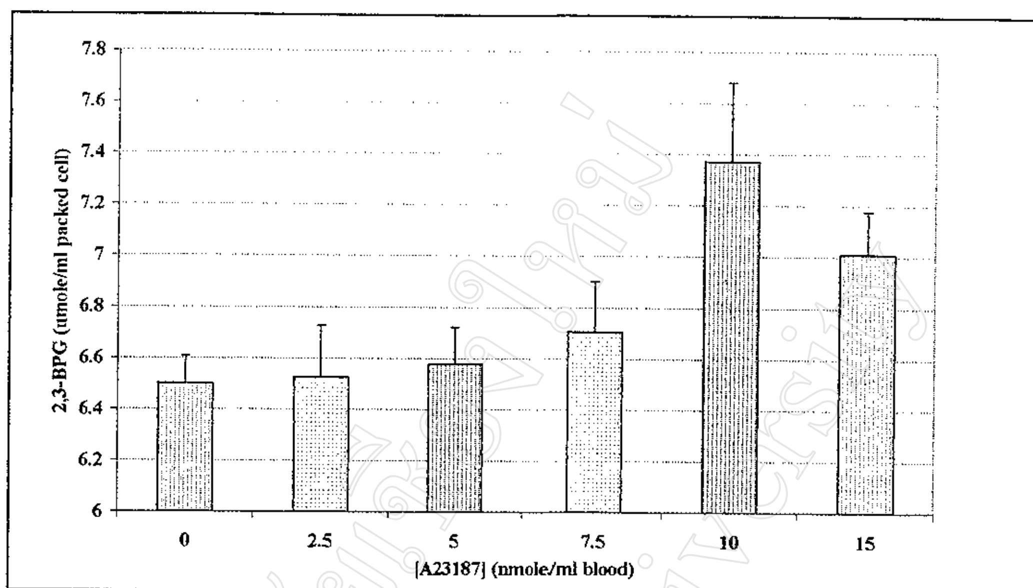

Figure 3-5: The effects of A23187 on erythrocyte 2,3-BPG levels at pH 7.40, 37 $^{\circ}$C for 15 minutes. The levels of erythrocyte 2,3-BPG were expressed as mean $\pm$ SD (n=5).

3.4.4 Effects of pO_2 on erythrocyte 2,3-BPG levels.

2,3-BPG plays an important role in oxygen transport and oxygen delivery by facilitating oxygen release from oxyhemoglobin. During exercise, increased oxygen delivery to active muscle is required to provide continuous aerobic energy metabolism. The resulting rise in oxygen consumption decreases pO_2 in the capillaries of active muscle tissue capillaries. Thus, pO_2 may affect the change in the metabolism of erythrocyte 2,3-BPG during exercise. To investigate the correlation between erythrocyte 2,3-BPG levels and pO_2, the blood was drawn from normal male Wistar rat and collected in potassium oxalate containing tube. The erythrocytes were washed three times with normal saline and resuspended in HBSS pH 7.4 at approximately 30% hematocrit. The erythrocyte suspension was filled in a screwed cap tube. Each suspension was blown with nitrogen gas and incubated at 37°C for 15 minutes. After that, the erythrocyte 2,3-BPG levels and pO_2 were immediately determined. These results were shown in Table 3-11 and Figure 3-6. There was a trend towards an increase in erythrocyte 2,3-BPG level as the pO_2 of the suspension was decreased. From these results, it can be concluded that pO_2

affect the change in 2,3-BPG metabolism. A marked decrease in pO_2 in active muscle tissue capillaries may be a factor that caused the 2,3-BPG levels to increase in erythrocyte.

Table 3-11: The effects of pO_2 on erythrocyte 2,3-BPG levels at pH 7.40, 37°C for 15 minutes.

pO_2 (mmHg)	2,3-BPG (μmole/ml packed cells)
127.90	7.23
154.30	6.96
156.10	6.96
158.20	6.56
161.00	6.16
162.00	7.10
174.70	6.69
174.90	6.43
183.30	6.43
208.10	6.02

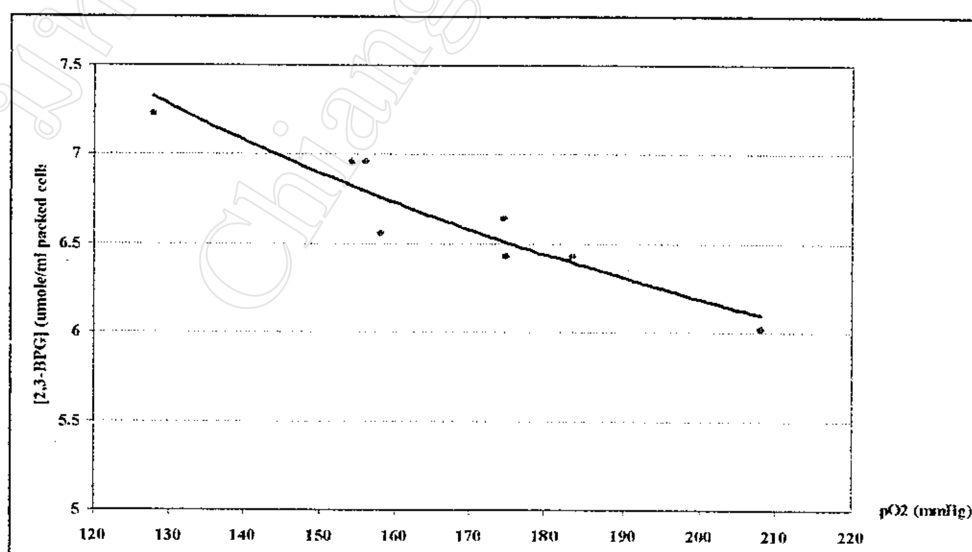

Figure 3-6: The effects of pO_2 on erythrocyte 2,3-BPG levels at pH 7.40, 37°C for 15 minutes.

3.4.5 Effects of pH, temperature, A23187 and pO_2 on the quantity of deoxyhemoglobin.

2,3-BPG, only bound to deoxyhemoglobin, it is physiologically important by facilitating oxygen release from oxyhemoglobin as red cells are passing through tissue capillaries. Since the rise in erythrocyte 2,3-BPG levels happens by acidic pH, an elevated temperature, an excess Ca^{2+} and a marked decrease in pO_2, deoxyhemoglobin is another factor that may also causes the rise in erythrocyte 2,3-BPG levels during exercise. To investigate that the rise in erythrocyte 2,3-BPG levels may be due to the rise in deoxyhemoglobin during incubation at pH 7.2, 43°C, an excess Ca^{2+} induced by 10 nmole of A23187 or the depletion of pO_2, blood was drawn from normal male Wistar rat and collected in potassium oxalate containing tube. The washed red cells were resuspended in HBSS pH 7.4 at approximately 5% hematocrit. The red cell suspension was filled in a screw cap tube and blowed with nitrogen gas for 5-15 minutes, matching with the suspension which was not nitrogen gas blowing. All suspensions were incubated at 37°C for 15 minutes. To evaluate the effect of temperature at 43°C on the quantity of deoxyhemoglobin, the red cell suspension with HBSS pH 7.4 were filled in a screw cap tube and then, incubated at 43°C and 37°C for 15 minutes to serve as experimental and control system, respectively. In excess Ca^{2+} system, 0.5 ml of red cell suspended in pH 7.4 was added in a screw cap tube that containing 5 μl of 1.0 mM A23187 and not containing A23187 to serve as control and then, all tubes were placed to incubate at 37°C for 15 minutes. To obtain an acidic incubation, the washed red cells were resuspended with HBSS pH 7.2 and matching with the suspension with HBSS pH 7.4 to serve as control system. Then the suspension was filled in a screw cap tube to incubate at 37°C for 15 minutes. Immediately after incubation, 0.1 ml of incubated red cells suspension was added into cuvette containing 0.9 ml of distillated water. Then, the quantitative estimation of oxyhemoglobin and deoxyhemoglobin were performed by scanning spectrophotometric method. Oxyhemoglobin and deoxyhemoglobin have distinctive absorption spectra, deoxyhemoglobin shows a single band at 552.5 nm, whereas oxyhemoglobin shows two bands at 541.5 and 576 nm (White *et al.,* 1968). In this study, the absorption spectra were scanned at the wavelength between 300 and 700 nm. The results were shown in Figure 3-7 and Figure 3-8. The absorption

spectrum obtained from red blood cells incubating under nitrogen gas was shown in Figure 3-7 (b), two bands of oxyhemoglobin at 576.40 and 541.60 nm were observed, whereas the single band of deoxyhemoglobin was not appear in this part of spectrum. The absorption spectrum from control system in Figure 3-7 (a) also showed 2 bands of oxyhemoglobin at 576.80 and 541.60 nm. Likewise, the absorption spectrum from rat erythrocyte incubated at $43^{\circ}C$ showed only two bands of oxyhemoglobin (Figure 3-8 (b)) and it was not different from control system (Figure 3-8 (a)). Moreover, the two bands of oxyhemoglobin also observed when rat erythrocytes were incubated at pH 7.2 and in the excess of Ca^{2+} ion. From these results, it can be confirmed that a marked increase in erythrocyte 2,3-BPG levels induced by each of several factors that occurred during exercise does not correlate to the rise in deoxyhemoglobin. Thus, a marked decrease in pO_2, temperature at $43^{\circ}C$, an excess Ca^{2+} induced by 10 nmole of A23187 and pH 7.2 are all factors that caused the 2,3-BPG levels to increase in erythrocyte.

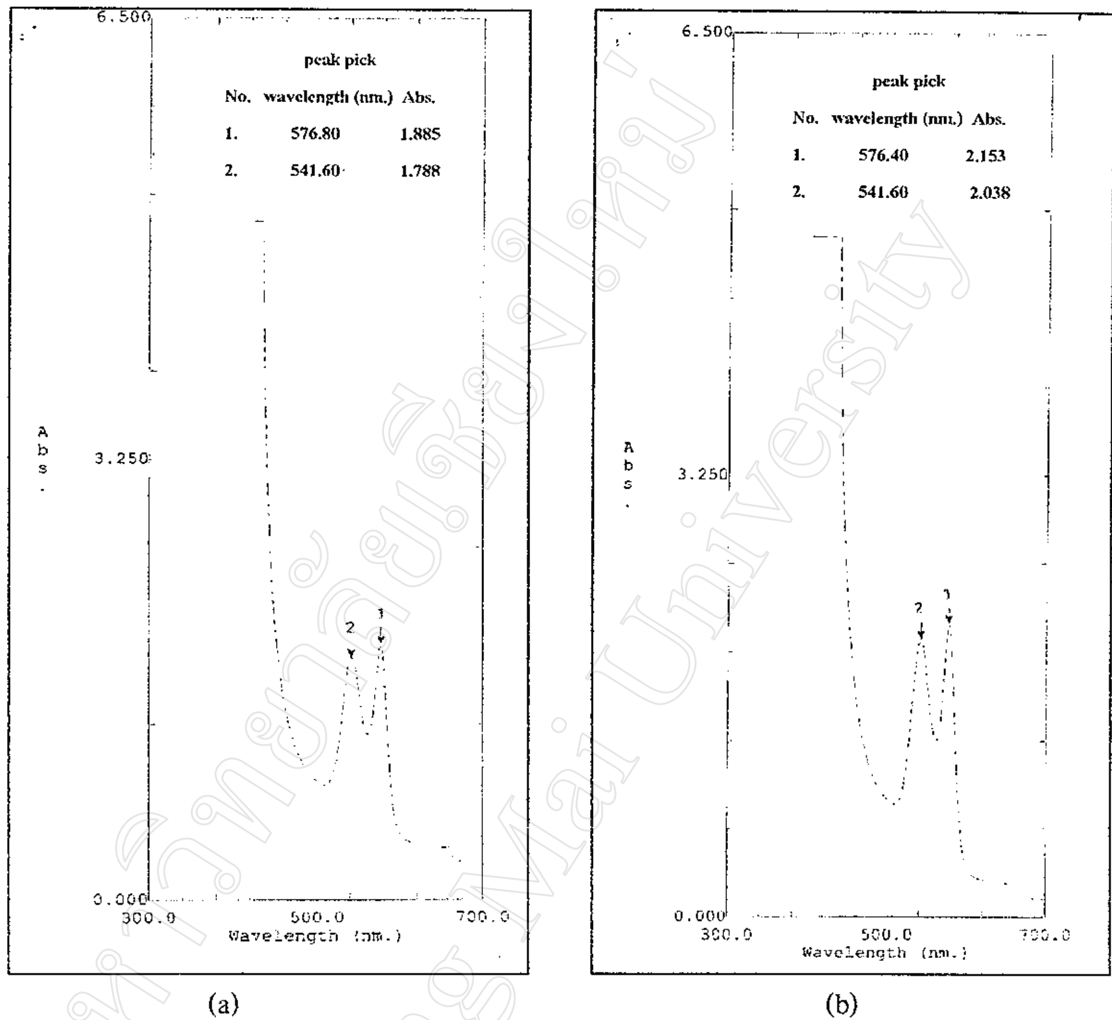

Figure 3-7: Absorption spectrum of rat hemolysate from incubation at atmospheric pO_2 , 37°C, 15 minutes (a) showing two bands of oxyhemoglobin at 576.80 and 541.60 nm. ; (b) incubation under nitrogen gas, 37°C for 15 minutes showing two bands of oxyhemoglobin at 576.40 and 541.60 nm.

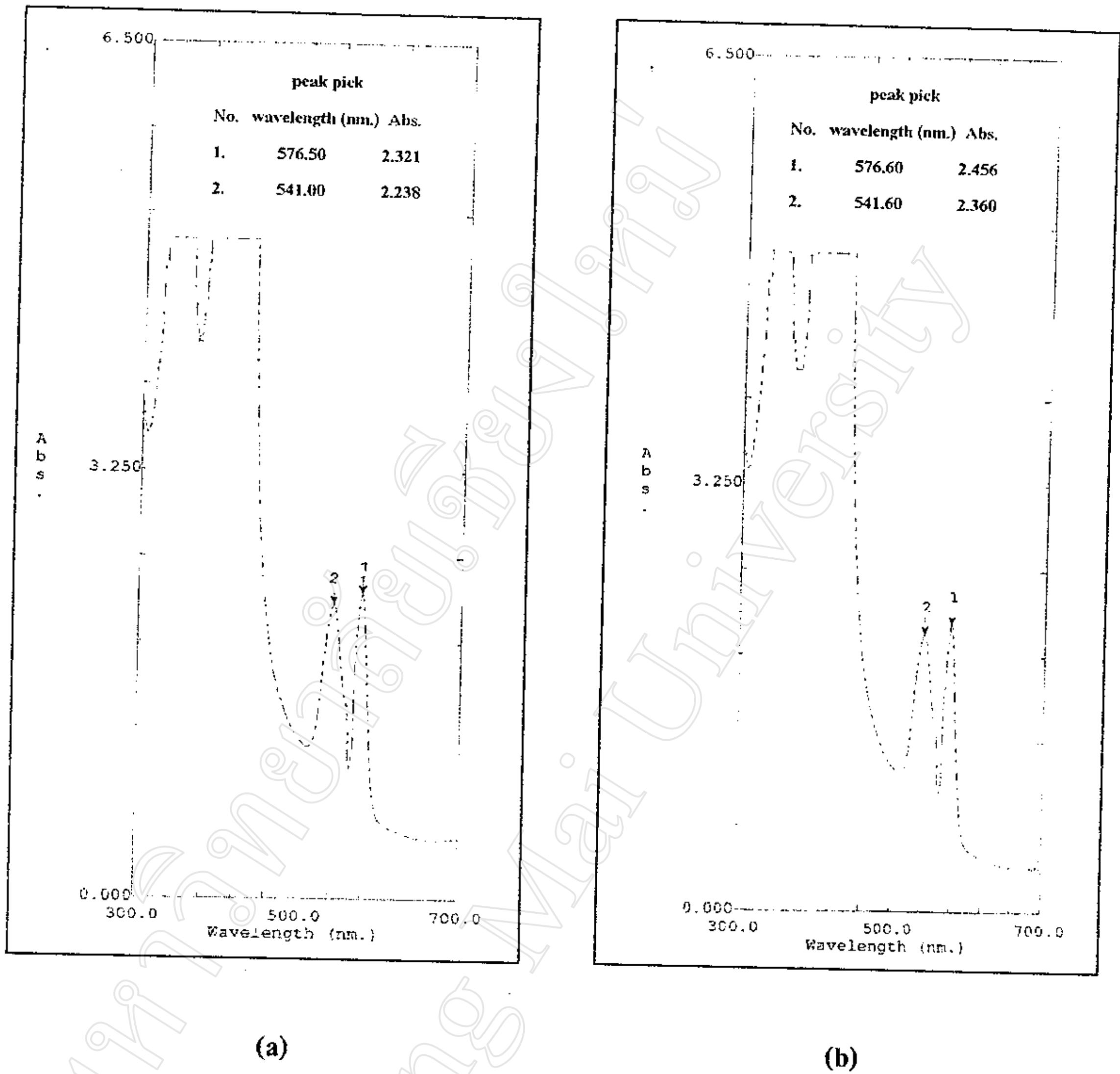

Figure 3-8: Absorption spectrum of rat hemolysate from incubation at 37°C for 15 minutes atmospheric pO_2 (a) , showing two bands of oxyhemoglobin at 576.50 and 541.00 nm ; (b) incubation at 43°C for 15 minutes atmospheric pO_2, showing two bands of oxyhemoglobin at 576.60 and 541.60 nm