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THE EFFECTS OF TEMPERATURE ON BLEEDING TIME AND CLOTTING TIME IN NORMAL VOLUNTEERS

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ABSTRACT

BACKGROUND: Bleeding time as a predictor of the potential for a bleeding disorder has been debated. The bleeding time measurement is known to be influenced by platelet count, mean platelet volume, platelet mass, von Willebrand's factor, factor VIII clotting protein, capillary integrity, vessel reactivity, and certain drugs, as well as by mechanical events associated with local perfusion pressure, resistance and blood flow. A bleeding time of less than 10 minutes has been arbitrarily established as a normal value.

In this study , the effects of temperature on bleeding time and clotting time were evaluated in healthy volunteers. Previous studies in humans subjected to extracorporeal bypass surgery and in baboons revealed correlations among increased bleeding time, reduced local skin temperature, and a reduced thromboxane B2 level in shed blood collected at the template bleeding time site.

METHODS: Forty-one (41) normal volunteers (19 males and 22 females) were subjected to local warming and cooling of the forearm to achieve local skin temperatures of +38C, +35C, +32C, +29C, +26C, +23C, and +20C or +37C, +32C, +28C, and +22C. Bleeding times were measured, and thromboxane B2 was measured in shed blood collected at the template bleeding time site. Thromboxane B2 is the stable metabolite of thromboxane A2 which vasoconstricts the blood vessels and aggregates the platelets at the bleeding time site. The total hemoglobin on the filter paper was recovered and measured, and the measurement was correlated to the bleeding time. Thromboxane B2 levels also were measured in the serum and plasma obtained from the venous blood collected from the antecubital vein of the locally cooled and warmed forearm.

Twenty-four (24) in vitro studies were done in blood collected from normal volunteers without altering the temperature of the forearm. In vitro clotting times were measured at +37C, +32C, +28C, and +22C and the serum was collected at the end of the clotting time or at 30 second intervals during the clotting of the blood for serum thromboxane B2 measurement in both agitated and non agitated blood.

We also studied the in vitro effects of temperature on the measurement of PT, PTT, factor V, and factor VIII and the in vitro effects of temperature on platelet aggregation and on thromboxane B2 production in platelet rich plasma isolated from venous blood collected from the antecubital vein after stimulation with a combination of arachidonic acid (AA) and adenosine diphosphate (ADP) or with ristocetin alone.

RESULTS: A reduction in local skin temperature from +38C to +20C was associated with a significantly increased bleeding time, a significantly decreased thromboxane B2 level in the shed blood collected at the bleeding time site, and a 15% reduction in the rate of thromboxane B2 production in the shed blood collected at the template bleeding time site. A reduction in the temperature of the forearm from +38 to +20C was associated with a significant reduction in the serum thromboxane B2 level in clotted blood obtained from the antecubital vein. The partial thromboplastin time and the prothrombin time were significantly increased when measured at +22C compared to +37C.

A prolonged bleeding time was associated with a significant increase in the total amount of hemoglobin recovered from the filter paper used to collect the blood from the bleeding time incision.

CONCLUSIONS: In healthy human volunteers, a reduction in skin temperature of the forearm was associated with a significant increase in bleeding time, a significant reduction in the thromboxane level in the shed blood obtained at the template bleeding site and a significant reduction in the serum thromboxane B2 level in the clotted blood obtained from the cooled forearm. These data indicate the importance of measuring local skin temperature at the time the bleeding time is performed, and of correcting the bleeding time measurement for the skin temperature. The bleeding time should be corrected for the skin temperature to distinguish the effects of the individual's platelets, clotting proteins, and vessel reactivity, as well as of drugs, anesthesia, and blood products on the bleeding time.

The temperature at which blood is clotted also affects clotting time: a reduction in temperature was associated with an increase in clotting time and a reduction in serum thromboxane B2 obtained from the clotted blood. Each degree centrigrade reduction in temperature was associated with approximately a 15 percent reduction in the rate of thromboxane B2 production during the clotting of the blood in vitro.

Hypothermic cardiopulmonary bypass patients and hypothermic surgical and trauma patients should be rewarmed post-operatively to improve platelet function and to reduce bleeding time and blood loss. In hypothermic bleeding patients who are to receive homologous blood products, both core and peripheral skin temperatures should be rewarmed to normal prior to transfusion. Rewarming is the safest and least expensive method of treating hypothermic patients with non-surgical blood loss, especially in view of the serious potential risks associated with homologous blood products.

INTRODUCTION

When normal healthy baboons were exposed to systemic and local hypothermia, they exhibited a hypothermia-induced reversible platelet dysfunction: a reduced local skin temperature was associated with a prolonged bleeding time accompanied by a reduction in thromboxane B2 in shed blood collected at the template bleeding time site.[1] Restoration of the baboon's local skin temperature to normal resulted in restoration to normal of both the bleeding time and the level of thromboxane B2 in shed blood.

Studies in patients undergoing hypothermic cardiopulmonary bypass surgery showed a correlation between non-surgical blood loss within the first 4 hours after surgery and the bleeding time measurement made 2 hours after surgery[2]. During the cardiopulmonary bypass period, a reduction in local skin temperature was associated with an increase in bleeding time and a reduction in the level of thromboxane B2 in the shed blood collected at the template bleeding time site. During the 2-hour to 24-hour post-operative period, the bleeding time decreased, the shed blood thromboxane B2 level increased, the mean platelet volume increased, and the local skin temperature at the site of the bleeding time measurement increased. In the 2-to 24-hour postoperative period during which bleeding time was reduced these patients exhibited a release of large platelets into the circulation and an increase in skin temperature at the template bleeding time site.

The effect of local skin temperature in hypothermic cardiopulmonary bypass surgery patients and its effect on the bleeding time were studied. One of the arms of the hypothermic patient was warmed and the other arm cooled.Bleeding times were measured on both arms and the shed blood was collected from the bleeding time incisions and assayed for thromboxane B2 [3]. A 6C reduction in the local skin temperature of one arm resulted in an increase in the bleeding time of 3 minutes and a significant reduction in the shed blood thromboxane B2 level.

We assessed the effects of temperature on the bleeding time in normothermic male and female volunteers at local skin temperatures ranging from +38C to +20C. The shed blood from the bleeding time incision was collected and assayed for thromboxane B2. In blood that was clotted with and without agitation at temperatures ranging from +38C to +20C thromboxane B2 was measured in the serum obtained from the clotted blood.

Studies were done to assess the effect of aggregating platelets in vitro at +37C and +22C on the magnitude of aggregation and the ability of the platelet to produce thromboxane after stimulation with either a combination of arachidonic acid (AA) and adenosine diphosphate (ADP) or with ristocetin alone were measured.

MATERIAL AND METHODS

Forty-one healthy volunteers, 19 males and 22 females between the ages of 19 to 29, participated in this study which was approved by the Institutional Review Board at Boston University School of Medicine. Each volunteer signed an informed consent form. These volunteers were taking no medication.

Sixteen volunteers, eight (8) male and eight (8) female were studied at four measured local skin temperatures of +22C, +28C, +32C, and +37C. Ten volunteers five, (5) males and five (5) females were studied at seven measured local skin temperatures of +20C; +23C; +26C; +29C; +32C; +35C; and +38C. To assess three methods to collect the shed blood at the template bleeding time site nine volunteers, (6) females and (3) males were studied at a measured local skin temperature of +32C, and six volunteers (3) males and (3) females were studied at three measured local skin temperatures of +22C, +28c, and +32C. The forearm skin temperature of the volunteers was equilibrated to temperatures between +20C and +38C by one or more of the following methods: a stream of air cooled by dry ice or wet ice, a walk-in 4C cold room, a hair dryer, and a heating lamp. Local skin temperature was monitored at thirty second intervals by a surface thermometer (Skin Temperature Sensor, Mon-A-Therm, Inc., St Louis, MO) placed within a few millimeters of the bleeding time site. Duplicate vertical bleeding times were measured according to the method of Babson and Babson[4].

Each bleeding time procedure produced two skin incisions. In all forty-one studies one template bleeding time was used for measurement of the bleeding time: the mean bleeding time of the two skin incisions is reported. In sixteen studies the blood collected on the filter paper during the bleeding time procedure was recovered and the total hemoglobin was measured using the cyanmethemoglobin method: the total hemoglobin is reported in milligrams.

In sixteen studies the shed blood from a second template bleeding time was collected to measure thromboxane B2. Blood emerging from the bleeding time was collected at thirty second intervals with a blunt end needle attatched to a 1 ml syringe containing heparin (1000 U/ml) and 40 lambda of ibuprofen (1.9mg/ml) until a volume of 600 lambda was collected. In the same sixteen studies where the local skin temperature was +37C, +32C, +28C, and +22C peripheral venous blood samples were collected from the antecubital vein of the locally cooled or warmed forearm. These blood samples were collected into tubes coated with heparin (1,000 U/ml; USP) and containing 40 lambda of ibuprofen (1.9 mg/ml), and were kept on wet ice until the blood was centrifuged at 1650 X g (3000 RPM) in a Sorvall GLC-3 centrifuge for 10 minutes; the plasma was removed and frozen and stored at -80C. Thromboxane B2 levels were measured on the thawed samples. Blood samples were also collected and allowed to clot without agitation at room temperature, and the serum was removed and frozen at -80C. Thromboxane B2 level measurements were made on the thawed samples. Blood samples from the same sixteen volunteers were collected for measurement of hemoglobin concentration (gm%), hematocrit (V%), white blood cell count (#/ul), platelet count (#/ul), and the mean platelet volume (MPV u^3) using the Coulter Counter JT Instrument. In addition, partial thromboplastin time (PTT/seconds), prothrombin time (PT/seconds), and factors V and VIII clotting proteins (percent of normal) were measured in vitro at +37C and +22C in blood samples collected in sodium citrate: the ratio was one volume of 3.8% sodium citrate to 9 volumes of blood[5]. Platelet aggregation and the production of thromboxane B2 in platelet rich plasma prepared from sodium citrated blood stimulated with a combination of arachidonic acid (0.05 mg/ml AA) and adenosine diphosphate (0.01 mM ADP), and ristocetin alone (1.25 mg/ml) were measured. The area under the aggregation curve at the five minute time point was measured by a digitizer and reported as digitizer units/5 minutes. Five minutes after stimulation of the platelets with AA and ADP thromboxane B2 production was stopped by the addition of 1 mg/ ml ibuprofen. The sample was centrifuged at 1600 x g for 10 minutes, and the plasma was frozen and stored at -20C until the thromboxane B2 assay was done. The thromboxane B2 per 10^{-5} platelet is reported.

In nine studies the shed blood emerging from the bleeding time site was collected for thromboxane B2 measurement using three different methods at a local skin temperature of +32C as follows: (a) at thirty second intervals blood was collected into heparin (1000 u/ML) and ibuprofen (1.9 MG/ML) until a volume of 600 lambda was collected; (b) all the shed blood was collected from one bleeding time site at thirty second intervals into heparin and ibuprofen; and (c) shed blood was collected at 2-minute intervals for the duration of the bleeding time. In six studies shed blood was collected using these three above mentioned methods at local skin temperatures of +22C, +28C, and +32C. Shed blood was kept on ice until it was centrifuged at 1650 X g (3000 RPM) in a Sorvall GLC-3 centrifuge for 10 minutes, the supernatant was removed and was frozen and stored at -80C until the measurements were made. Thromboxane B2 measurements were done on the thawed samples using the thromboxane B2 (^{125}I) RIA Kits (New England Nuclear Corp., Boston, MA). In six studies the thromboxane B_2 production rate (pg/ml/second) during the bleeding time measurement was calculated from the thromboxane B_2 level in all the shed blood collected at the bleeding time site and the length of the bleeding time in seconds.

In five in vitro studies aggregation and thromboxane B2 production in response to a combination of AA and ADP or ristocetin alone at +37C and +22c was measured. In sixteen in vitro studies peripheral venous blood was collected for clotting time measurements at +22C, +28C, +32C, and +37C. Clotting times were done in 3.5 ml siliconized glass tubes with 1 or 3 ml of blood, or in 7 ml siliconized tubes with 7 ml of blood: the tube was agitated every 30 seconds until a clot was formed. Matched blood samples were allowed to clot non agitated at the same temperature in the same size tubes for the time required for the blood to clot in the tubes that were agitated. The serum was separated from all the clotted blood samples by centrifugation and was frozen at -80C. Thromboxane B2 measurements were done on the thawed samples. The thromboxane B2 production rate (pg/ml/second) during the clotting of the blood was calculated from the serum thromboxane level and the length of the clotting time in seconds in the agitated samples.

Three studies were done to assess thromboxane B2 levels at 30 second intervals during the clotting of blood at +37C, +32C, +28C, and+22C .

One ml samples were collected into 3.0 ml siliconized glass tubes and each blood sample was agitated every 30 seconds until the addition of ibuprofen to halt the production of thromboxane. Five one ml aliquots were studied at each temperature and ibuprofen added to each tube successively. The samples were centrifuged and the serum frozen at -80C until assayed for thromboxane B2 level.

The actual value of the bleeding time, serum, plasma, and shed blood levels of thromboxane B2, and the total hemoglobin on the filter paper were reported as well as the natural logarithm of each value. The hematocrit, hemoglobin concentration, red blood cell count, platelet count, white blood cell count, MPV, the bleeding time, serum, plasma and shed blood thromboxane B2 level, and total hemoglobin on the filter paper at each temperature were analyzed with a one-way analysis of variance (ANOVA). The paired Students t-test was utilized for comparison of the sample means when the ANOVA was significant ($p<0.05$)[6]. Correlations were done by linear regression analysis. Analyses were done using a statistical software package

(PRODAS, Conceptual Software, Inc., Houston, TX) in an IBM personal computer.

RESULTS

Tables 1 and 2 report measurements in peripheral blood samples collected from the antecubital vein of the locally cooled or warmed forearm for 16 of the 41 healthy volunteers in the study. Hematological measurements made at local skin temperatures of +37C; +32C; +28C; +22C showed no significant changes in Hct, Hgb concentration, RBC count, WBC count, platelet count, or MPV (Table 1). Partial thromboplastin time (PTT), prothrombin time (PT), factor V, and factor VIII assays were measured at +22C and +37C. The PTT and PT were significantly prolonged and the factor V significantly reduced at +22C compared to +37C (Table 2).

Table 3A and Figures 1 to 8 report bleeding times and the thromboxane B2 levels in shed blood collected at the template bleeding time site and in the heparin-ibuprofen plasma and serum samples from venous blood obtained from the antecubital vein of the locally cooled or warmed forearm. Bleeding times measured at local skin temperatures of +37C, +32C, +28C, and +22C showed a significant increase at +22C (22.5 $\pm$ 7.5 minutes) compared to +37C (5.8 $\pm$ 1.3

minutes). In the shed blood, the thromboxane B2 level was significantly decreased at +22C (240 $\pm$ 178 pg/0.1 ml) compared to the level at +37C (3034 $\pm$ 1555 pg/0.1 ml). There were no significant differences in the plasma thromboxane B2 levels throughout the 22C to 37C range. The serum thromboxane B2 level did show a significant decrease from 2286 $\pm$ 1697 pg/0.1 ml at +37C to 668 $\pm$ 717 pg/0.1 at +22C. Table 3B and Figures 9 to 16 report the natural logarithm of bleeding time and thromboxane B2 level in the shed blood collected at the template bleeding time site; in the heparin-ibuprofen plasma; and in the serum from venous blood obtained from the antecubital vein of the locally cooled and warmed forearm maintained at +37C, +32C, +28C, and +22C.

Tables 4A, 4B, 5A, 5B and Figures 17 to 32 report thromboxane B_2 levels in shed blood collected from the bleeding time site using three different methods at local skin temperatures of +32C, +28C, and +22C. A volume of 0.6 ml of shed blood was collected; all the shed blood from one bleeding time was collected; and the shed blood was collected at 2 minute intervals throughout the bleeding time measurement. The highest thromboxane B2 levels were seen in the samples collected during the last 2 minute collection period of the bleeding time measurement at temperatures of +32C and +28C (Tables 4A, 4B, 5A, and 5B). Reduction in the local skin temperature to 28C and 22C increased the bleeding time and reduced the thromboxane B2 level in shed blood collected from the bleeding time site (Tables 4A, 4B, 5A, and 5B, Figures 17 to 32).

Tables 6 to 13; 14A, 14B, 15A, 15B, Figures 33 to 44 report the clotting times and the serum thromboxane B2 levels obtained from blood clotted at +37C, +32C, +28C, and +22C with and without agitation. Agitation of the blood during clotting produced significantly higher serum thromboxane B_2 levels at +32C and +37C (Tables 14A AND 14B).

Table 16 and Figures 45, 46, 47, and 48 report the rate of thromboxane production and the natural logarithm of the rate of thromboxane B2 production and in shed blood collected from the bleeding site in a forearm where the local skin temperature was maintained at +22C, +28C, +32C, and +37C, and in blood clotted with agitation at +22C, +28C, +32C, and +37C. For each 1C decrease in temperature there was a 15% decrease in the rate of thromboxane B2 production in both the shed blood and the agitated clotted blood.

Tables 17A and 17B, Figures 49 and 50 report the bleeding times and total hemoglobin levels collected from the bleeding time on the filter paper at local skin temperatures of +38C, +35C, +32C, 29C, 26C, 23C, and +20C. Bleeding time was significantly increased at +20C (22 $\pm$ 5.0 minutes) compared to +38C (5.3 $\pm$ 1.5 minutes). A significant correlation ($r=0.352, p<0.001, n=94$) was observed between bleeding time and the total hemoglobin on the filter paper (Table 17A and 17B, Figure 49 and 50).

Tables 18A, 18B, 19A, and 19B report the aggregation patterns and thromboxane B2 levels in the platelet-rich plasma obtained from sodium citrate venous blood collected from the locally warmed and cooled forearm at temperatures of +37C, +32C, +28C, and +22C. The platelet-rich plasma was stimulated at +37C with either a combination of arachidonic acid (0.05 mg/ml AA) and adenosine diphosphate (0.01 mM ADP) or with 1.25 mg/ml or ristocetin alone. Comparison of 5 minute aggregation patterns showed no significant differences whether the stimulus was a combination of AA and ADP or ristocetin alone (Tables 18A and 18B). Thromboxane B2 production during the aggregation was halted after five minutes of aggregation with the addition of 1 mg/ml ibuprofen. Table 19A and 19B report that thromboxane B_2 production was significantly greater following stimulation of the platelets with a combination of AA and ADP than with ristocetin alone. The aggregation patterns in response to a combination of AA and ADP and to ristocetin alone were similar although the thromboxane B_2 production by the platelets was significantly greater in response to AA and ADP than to ristocetin alone.

Tables 20A and 20B report platelet aggregation and the platelet production of thromboxane B2 in vitro at +22C and +37C following stimulation with AA and ADP and to ristocetin alone . Both the platelet aggregation and the platelet production of thromboxane B2 were better at +37C than at +22C; but these differences were not statistically significant.

Table 21 reports measured bleeding times in 10 normothermic volunteers at the 7 skin temperatures.The bleeding times were corrected to a skin temperature of 35C using the empirically derived factor: (T - 35C)/20C + 1, where T was the measured skin temperature when T was less than 35C.

Figure 51 reports the mean and standard deviation of the bleeding times in the 10 volunteers in whom bleeding time was measured at each of the 7 temperatures and corrected by the above factor.

DISCUSSION

Many factors are known to influence the bleeding time measurement, e.g., platelet count, mean platelet volume, platelet mass, von Willebrand's factor, factor VIII clotting protein, capillary integrity, vessel reactivity, drugs which affect platelet function, and mechanical events related to local perfusion pressure, resistance, and blood flow[7-16].

Previous studies in baboons and in patients undergoing extracorporeal bypass surgery have shown increased bleeding times associated with reduced local skin temperatures and reduced levels of thromboxane B2, the stable metabolite of thromboxane B2, in shed blood collected at the template bleeding time site[1-3]. The shed blood level of 6-keto PGF1a, the stable metabolite of prostacyclin, did not appear to affect the bleeding time[1-3]. In the present study involving healthy male and female volunteers, a reduced local skin temperature was found to have a definite effect on bleeding time: when the local skin temperature was reduced from 35C to 22C, the bleeding time increased 3 to 4 times[17]. However when the local skin temperature was increased from 35 to 38C, no significant effect on the bleeding time was observed.

The thromboxane B2 level in the shed blood collected from the template bleeding time site was influenced by the local skin temperature; decrease in local skin temperature significantly reduced the shed blood level of thromboxane B2 and significantly increased the bleeding time. The thromboxane B2 level in the serum from the blood collected from the warmed or cooled forearm and allowed to clot at room temperature without agitation was also influenced by local skin temperature. Blood clotted without agitation at +22C, +28C, +32C, and +37C showed a non significant increase in the serum thromboxane B_2 level as the temperature increased. However, blood clotted with agitation at +22C, +28C, +32C, and +37C showed a significant increase in the serum thromboxane B_2 level as the temperature increased. The serum thromboxane B_2 levels in blood clotted with agitation at +32C and +37C were significantly higher than the serum thromboxane B_2 levels in blood clotted without agitaion at +32C and +37C. These data show that the serum thromboxane B_2 level is influenced by both temperature and agitation of the blood during the clotting time. The data show that rate of thromboxane B2 production decreased by 15% for each 1C decrease in temperature in both the shed blood and the agitated clotted blood.

Our data in healthy volunteers show that temperature plays an important role in bleeding time and clotting time measurements. Our results further underscore the necessity of measuring skin temperature when measuring bleeding time and of correcting the measured bleeding time for the measured skin temperature using the empirically derived factor: T - 35C/20C+1 where T is the measured skin temperature and when T is less than 35C.[17] Only by this method is it possible to learn whether the bleeding time measurement has been influenced by the individual's platelets, clotting proteins, capillary integrity, vessel reactivity and other mechanical factors and by drugs, anesthesia, and transfused blood products.

In patients undergoing hypothermic cardiopulmonary bypass surgery, non-surgical blood loss during the 4-hours post-operative period was found to correlate with the bleeding time 2 hours post-op[2].

In the healthy volunteers the bleeding time correlated with the total hemoglobin recovered from the filter paper used to measure the bleeding time. Unlike reports to the contrary,[18,19] our data in previous reports [2,3] and in this study show that bleeding time correlates to non-surgical blood loss. Our data also support that a reduction in non-surgical blood loss is best achieved by cooling the periphery of the bleeding site and warming the bleeding time site[20,21].

Prevention of a bleeding diathesis generally associated with resuscitation of hypothermic patients in hemorrhagic shock, can be achieved by rewarming the patients to restore both core and peripheral temperature to normal. This would ensure optimum function of the patient's platelets and clotting proteins. Rewarming is critical to improved platelet function and reductions in both bleeding time and blood loss in hypothermic cardiopulmonary bypass patients and in hypothermic surgical and trauma patients. In view of the serious potential risks associated with homologous blood products, rewarming is the safest treatment for hypothermic patients with non-surgical blood loss, as well as the least expensive.

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TABLE 1

THE HEMATOLOGIC MEASUREMENTS IN PERIPHERAL BLOOD COLLECTED FROM THE ANTECUBITAL VEIN OBTAINED FROM NORMAL MALE AND FEMALE VOLUNTEERS SUBJECTED TO LOCAL COOLING AND WARMING OF THE FOREARM TO ACHIEVE LOCAL SKIN TEMPERATURES OF +37, +32, +28, AND +22C

Temp	PLT Count ($x10^3$/ul)	HCT (V%)	HGB (GM%)	WBC Count ($x10^3$/ul)	RBC (x106/ul)	MPV (u^3)
37C						
Mean:	254	37	12	5.1	3.9	7.6
SD:	66	10	3	2	1.1	2.1
n:	15	16	16	16	16	16
32C						
Mean:	264	38	13	5.1	4.0	7.5
SD:	70	10	4	2	1.1	2.1
n:	15	16	16	16	16	16
28C						
Mean:	255	37	12	5.3	-3.9	7.9
SD:	69	10	3	2	1.1	2.2
n:	15	16	16	16	16	16
22C						
Mean:	262	36	12	6.0	3.9	7.7
SD:	69	10	3	2	1.1	2.2
n:	15	16	16	16	16	16
1 Way ANOVA:	ns	ns	ns	ns	ns	ns

TABLE 2

THE MEASUREMENT OF THE PTT, PT, FACTOR V AND FACTOR VIII CLOTTING PROTEINS AT 22C AND 37C IN SODIUM CITRATE PLASMA OBTAINED FROM NORMAL VOLUNTEERS AT A LOCAL SKIN TEMPERATURE OF +32C

n=4	37C	**Paired t TEST** Between 37C & 22C	22C
PTT (Sec)			
Mean:	46		95
SD:	2	<.001	52
PT (Sec)			
Mean:	17		40
SD:	1.5	<.001	2
Factor V (% of normal)			
Mean:	69		29
SD:	44	<.05	21
Factor VIII (% of normal)			
Mean:	54	NS	44
SD:	12		4

TABLE 3A

THE BLEEDING TIME AND THE LEVELS OF THROMBOXANE B_2 IN THE SHED BLOOD, THE VENOUS HEPARIN-IBUPROFEN PLASMA, AND VENOUS SERUM OBTAINED FROM NORMAL VOLUNTEERS SUBJECTED TO LOCAL COOLING AND WARMING OF THE FOREARM TO ACHIEVE LOCAL SKIN TEMPERATURES OF +37C, +32C, +28C, AND +22C

Temp		Bleeding Time (min)	Shed Blood TxB2 (pg/ 0.1 ml) in 0.6 ml of shed blood	Venous Heparin-Ibuprofen Plasma TxB2 (pg/ 0.1 ml)	Venous Serum TxB2 (pg/ 0.1 ml)
37C					
	Mean:	5.8	3034	31	2286
	SD:	1.3	1555	15	1697
	n:	16	15	16	16
32C					
	Mean:	6.3	2010	31	2174
	SD:	2	1357	16	1154
	n:	16	15	16	16
28C					
	Mean:	10.3	422	33	1678
	SD:	3	213	10	1008
	n:	16	15	16	16
22C					
	Mean:	22.5	240	28	668
	SD:	7.5	178	9	717
	n:	16	15	15	16
1-Way ANOVA:		<.001	<.001	ns	<.05

FIGURE 1

THE RELATIONSHIP BETWEEN THE SKIN TEMPERATURE AND THE BLEEDING TIME IN NORMAL VOLUNTEERS

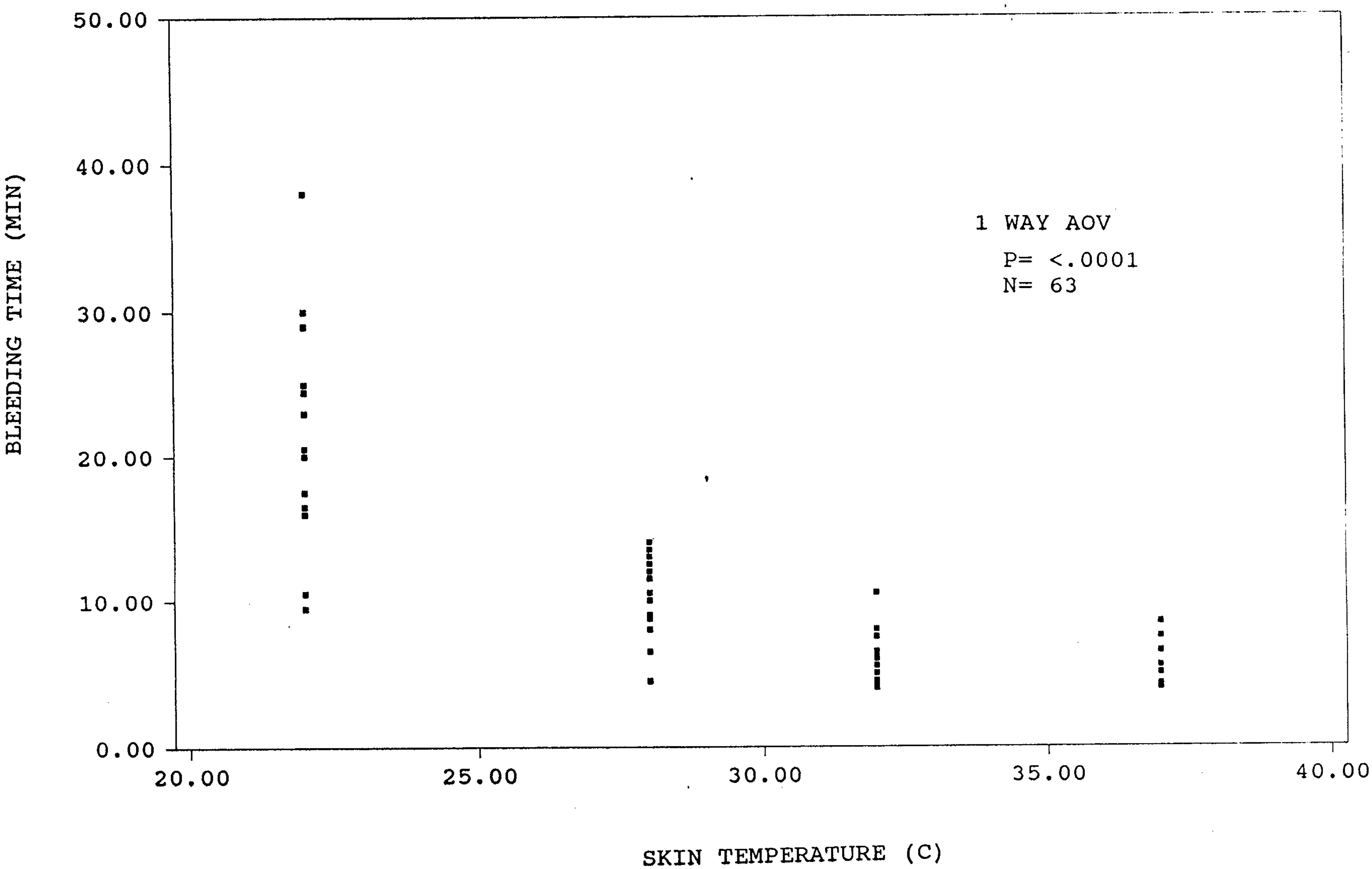

FIGURE 2

THE RELATIONSHIP BETWEEN THE SKIN TEMPERATURE AND THE SHED BLOOD THROMBOXANE B_2 LEVEL IN NORMAL VOLUNTEERS

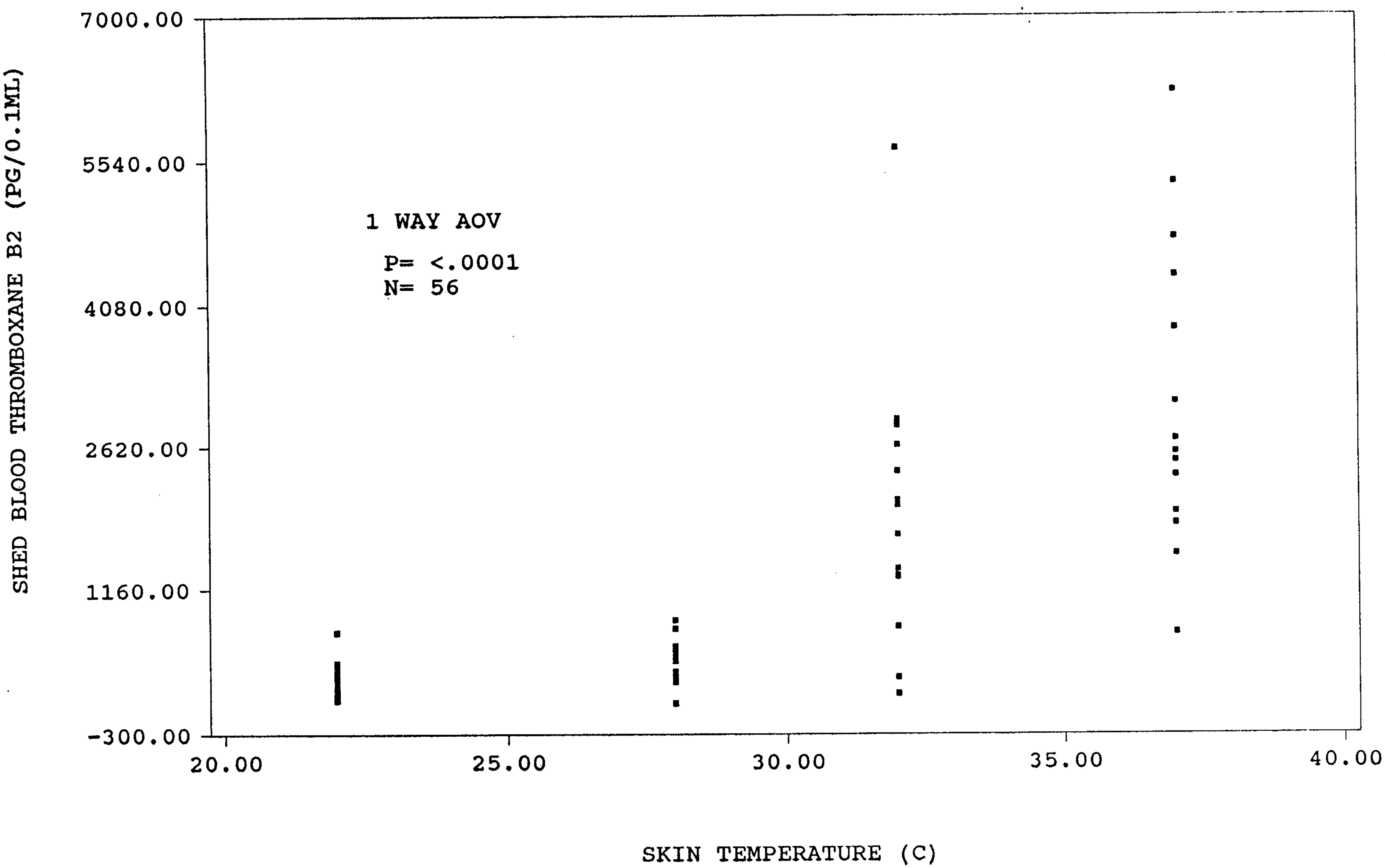

FIGURE 3

THE RELATIONSHIP BETWEEN THE SKIN TEMPERATURE AND THE PLASMA THROMBOXANE B_2 LEVEL IN NORMAL VOLUNTEERS

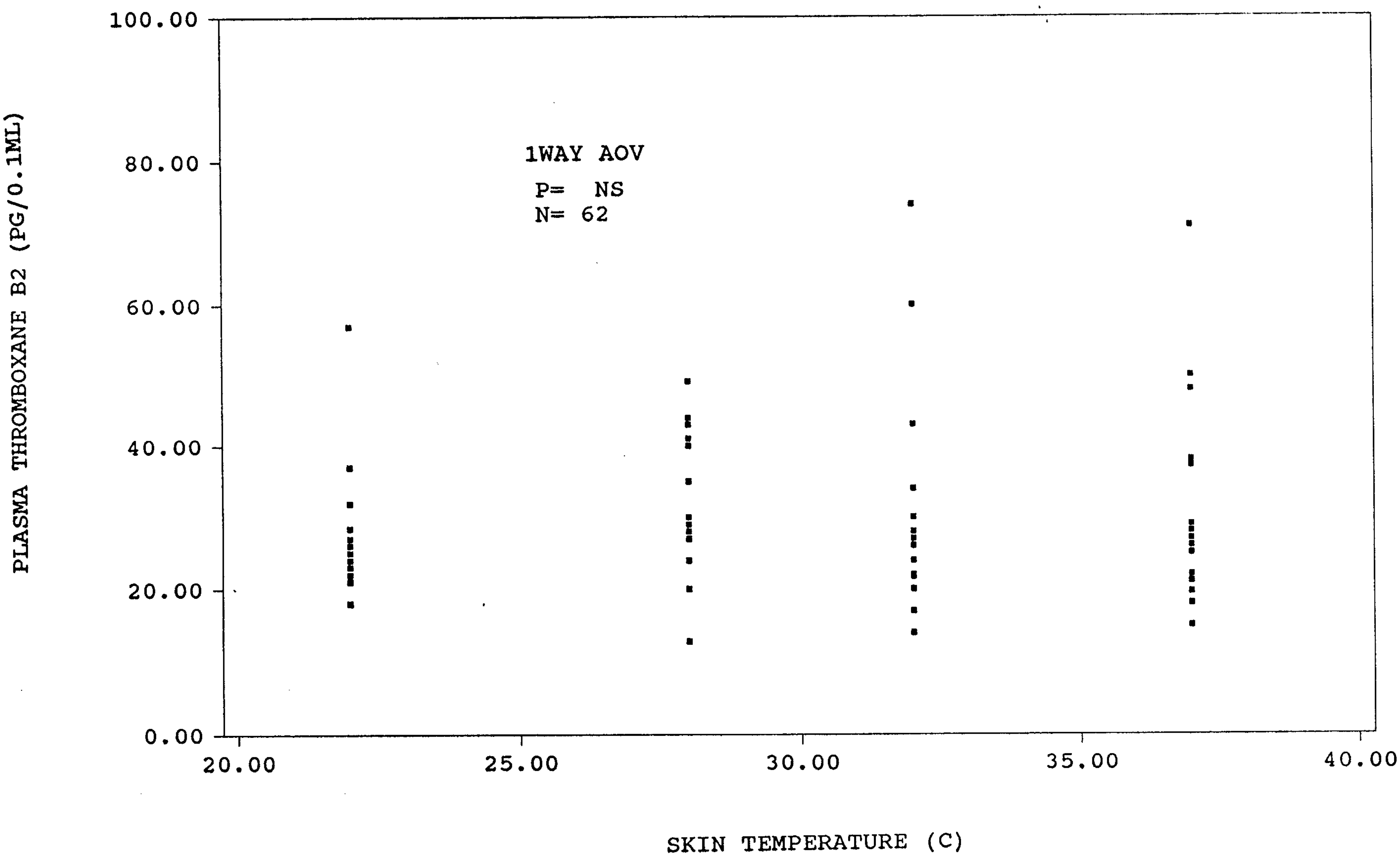

FIGURE 4

THE RELATIONSHIP BETWEEN THE SKIN TEMPERATURE AND THE SERUM THROMBOXANE B_2 LEVEL IN NORMAL VOLUNTEERS

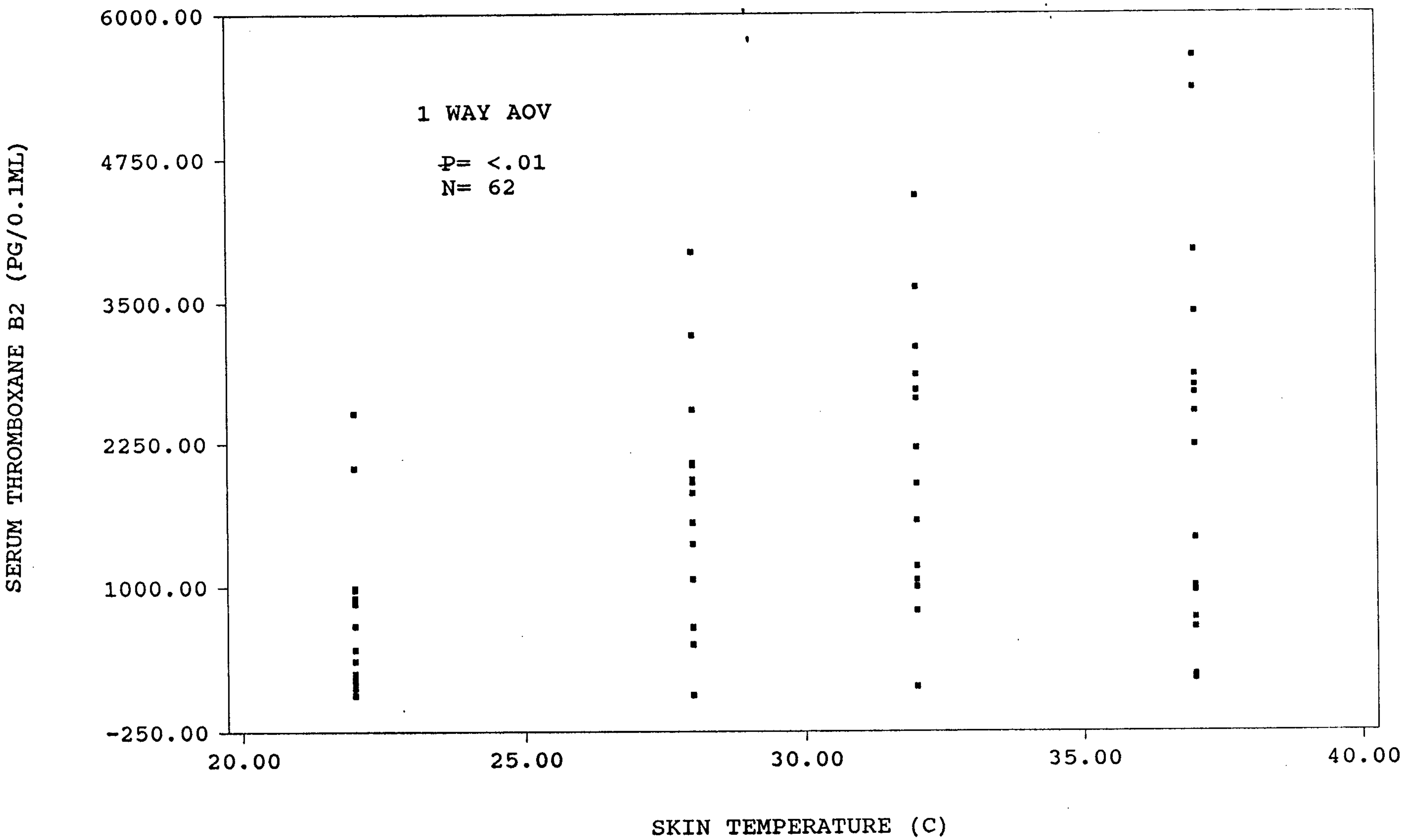

FIGURE 5

DISTRIBUTION OF THE BLEEDING TIME IN MINUTES IN NORMAL VOLUNTEERS

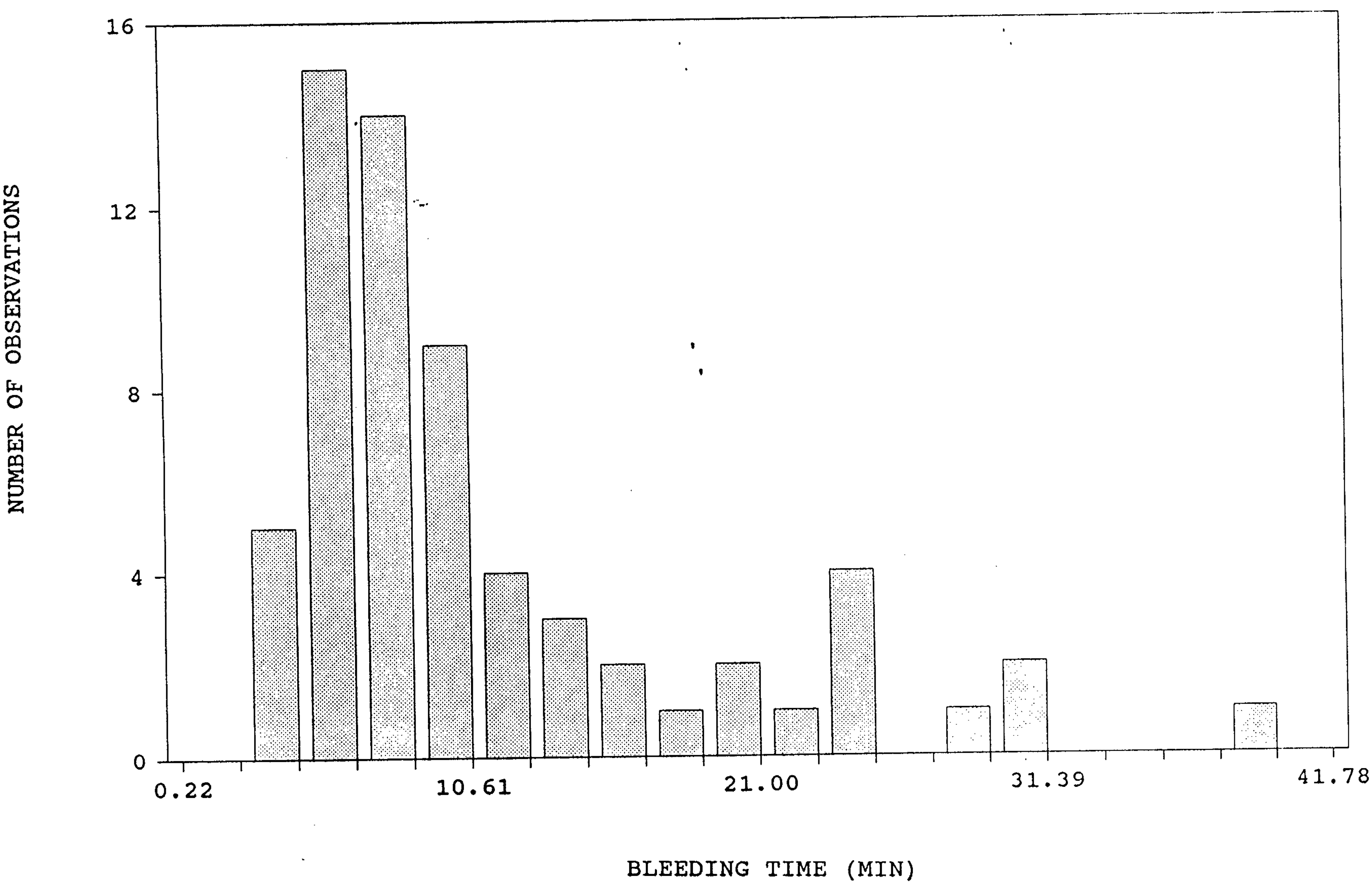

FIGURE 6

DISTRIBUTION OF THE SHED BLOOD THROMBOXANE B_2 LEVEL IN NORMAL VOLUNTEERS

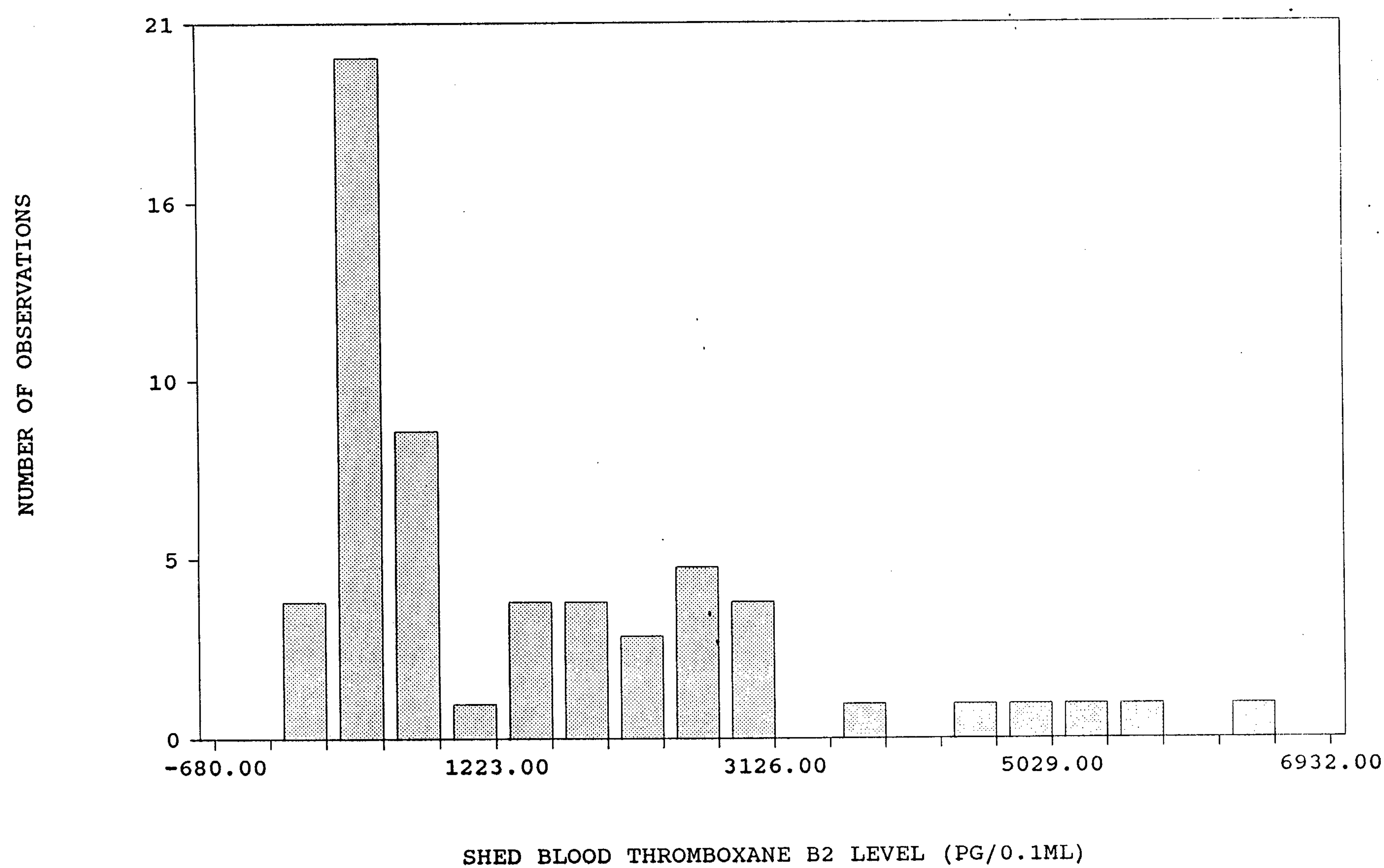

FIGURE 7

DISTRIBUTION OF THE PLASMA THROMBOXANE B_2 LEVELS IN NORMAL VOLUNTEERS

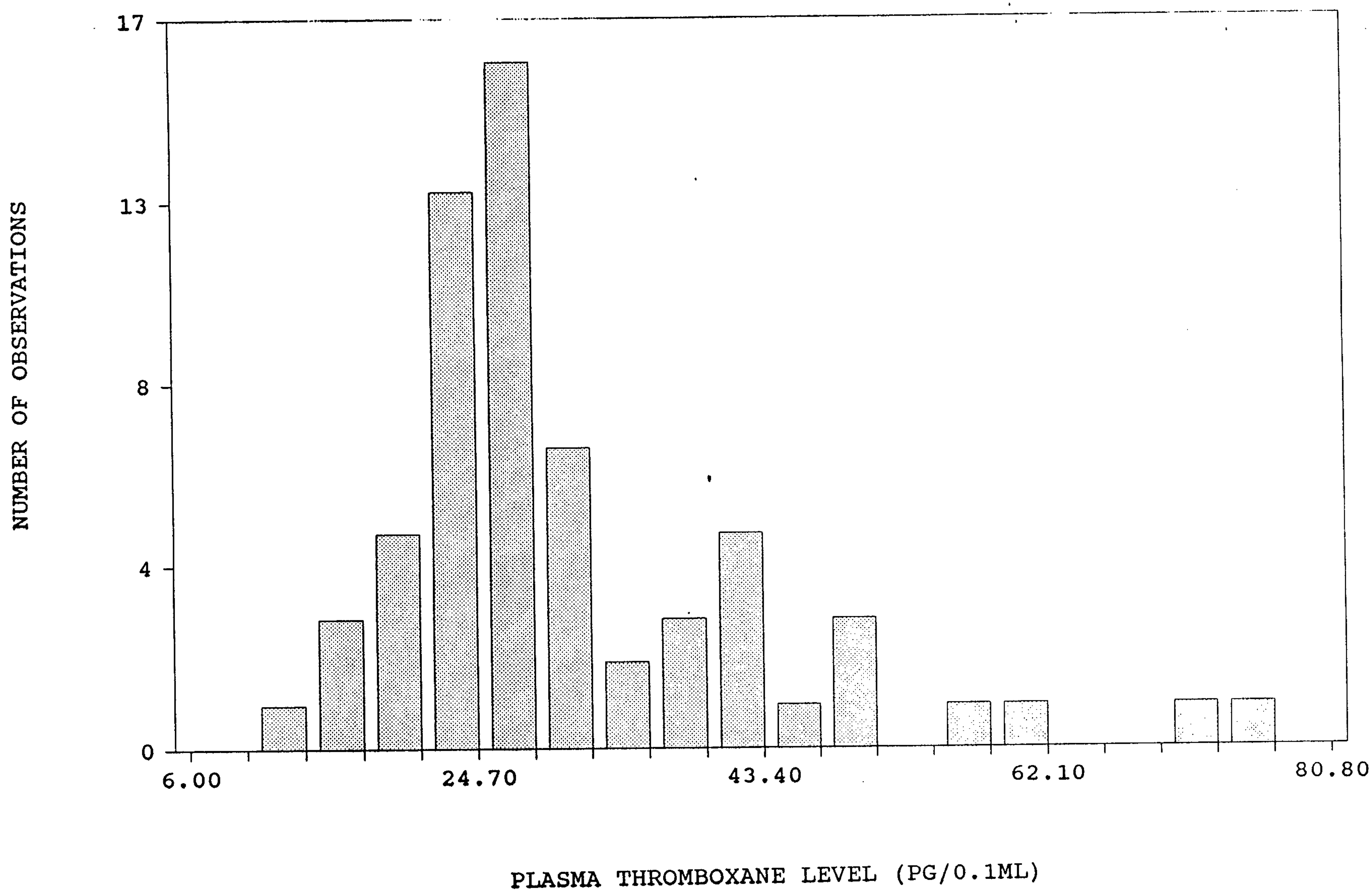

FIGURE 8

DISTRIBUTION OF THE SERUM THROMBOXANE B_2 LEVEL IN NORMAL VOLUNTEERS

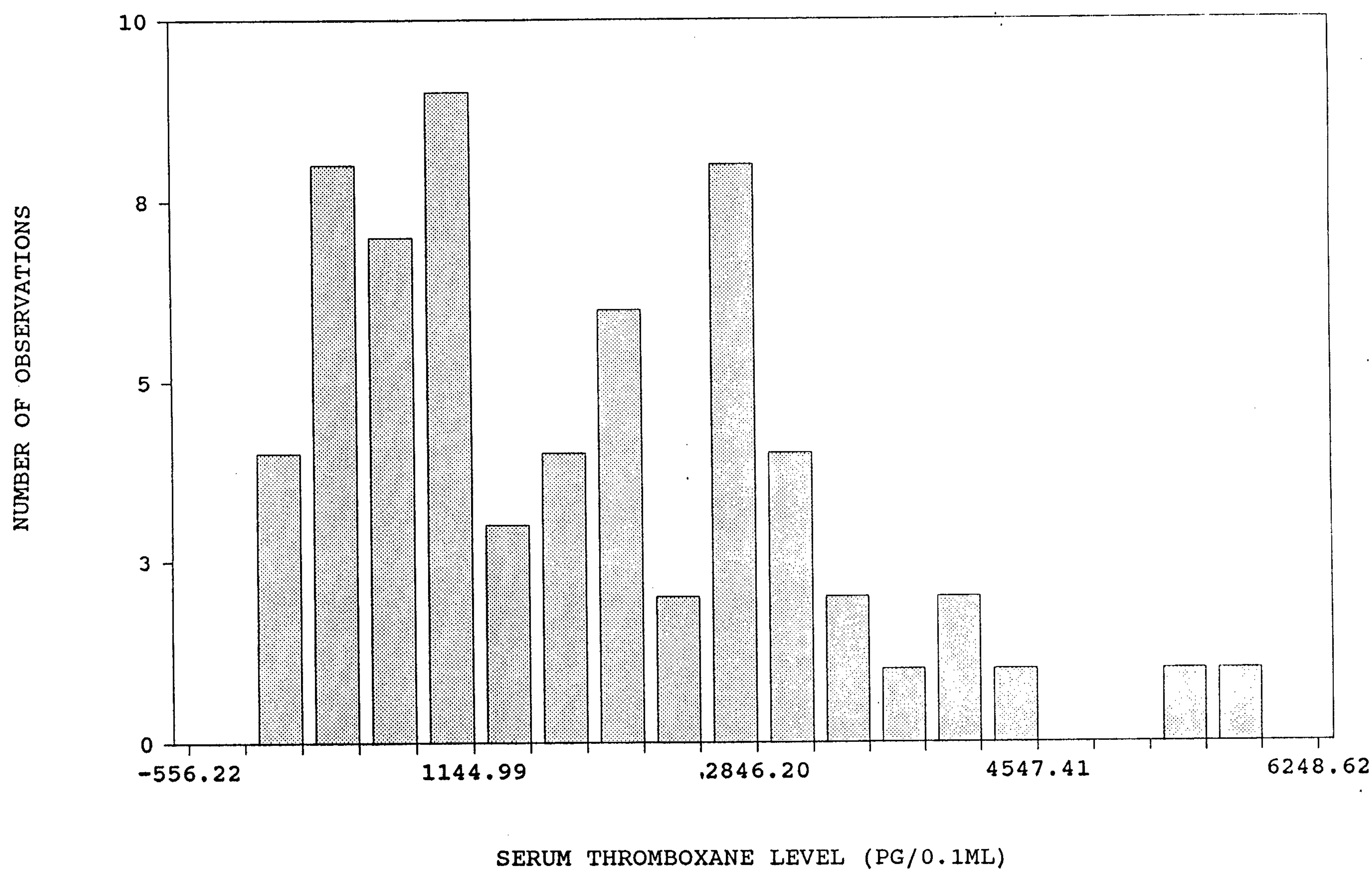

TABLE 3B

THE NATURAL LOGARITHM OF THE BLEEDING TIME AND THE LEVELS OF THROMBOXANE B_2 IN THE SHED BLOOD, THE VENOUS HEPARIN-IBUPROFEN PLASMA, AND VENOUS SERUM OBTAINED FROM NORMAL VOLUNTEERS SUBJECTED TO LOCAL COOLING AND WARMING OF THE FOREARM TO ACHIEVE LOCAL SKIN TEMPERATURES OF +37C, +32C, +28C, AND +22C

Temp	Bleeding Time (min)	Shed Blood TxB2 (pg/ 0.1 ml) in 0.6 ml of shed blood	Venous Heparin-Ibuprofen Plasma TxB2 (pg/ 0.1 ml)	Venous Serum TxB2 (pg/ 0.1 ml)
37C				
Mean:	1.73	7.8	3.28	7.36
SD:	.2	.5	.4	1.0
n:	16	15	16	16
32C				
Mean:	1.81	7.29	3.32	7.46
SD:	.3	1.0	.4	.8
n:	16	15	16	16
28C				
Mean:	2.3	5.80	3.44	7.14
SD:	.3	.9	.3	.9
n:	16	15	16	16
22C				
Mean:	3.05	5.21	3.28	6.07
SD:	.4	.8	.3	1.1
n:	16	15	15	15
1 Way ANOVA:	<.001	<.001	ns	<.001

FIGURE 9

THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE BLEEDING TIME AND THE SKIN TEMPERATURE

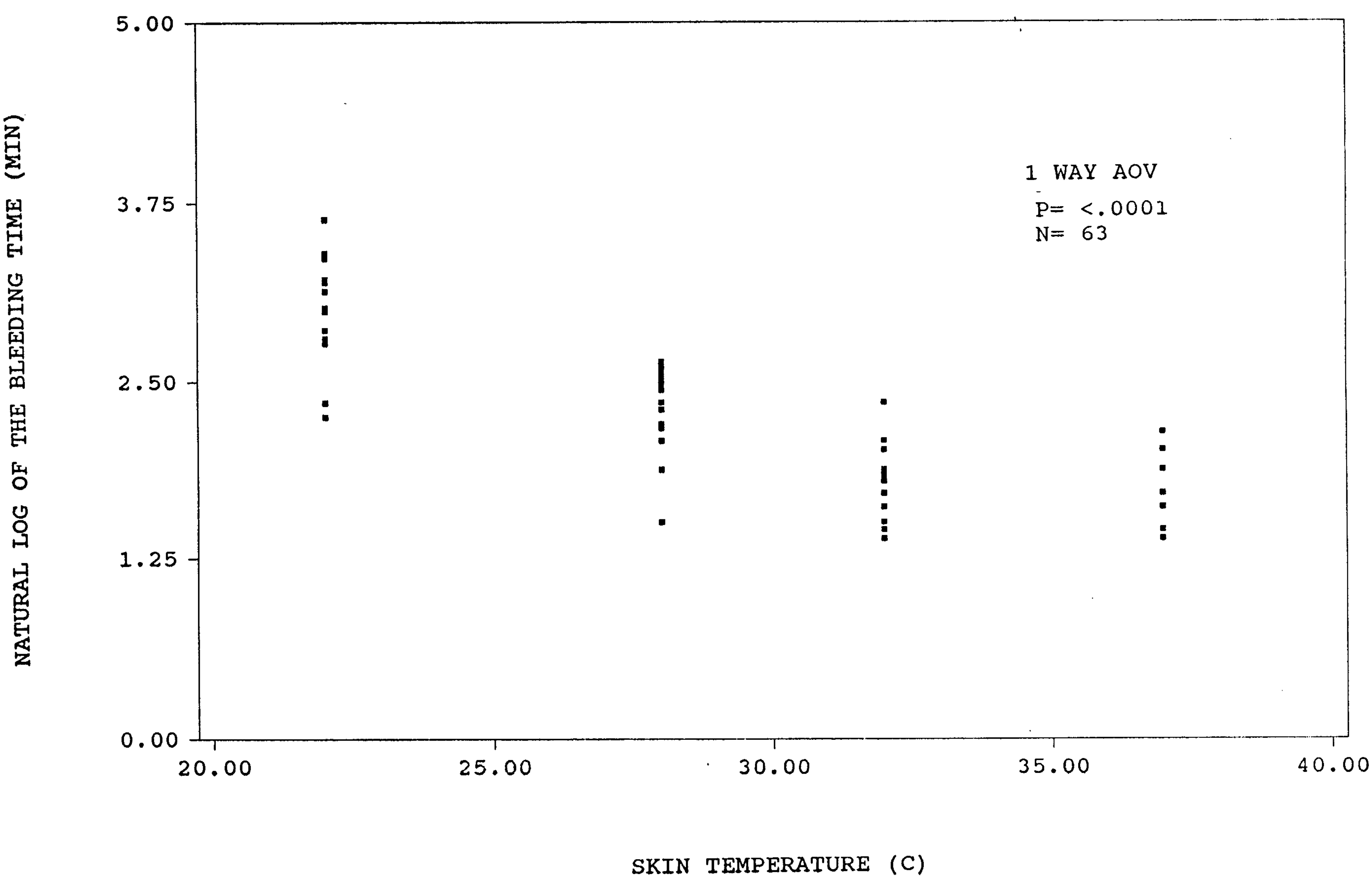

FIGURE 10

THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE SHED BLOOD THROMBOXANE B_2 LEVEL AND THE SKIN TEMPERATURE

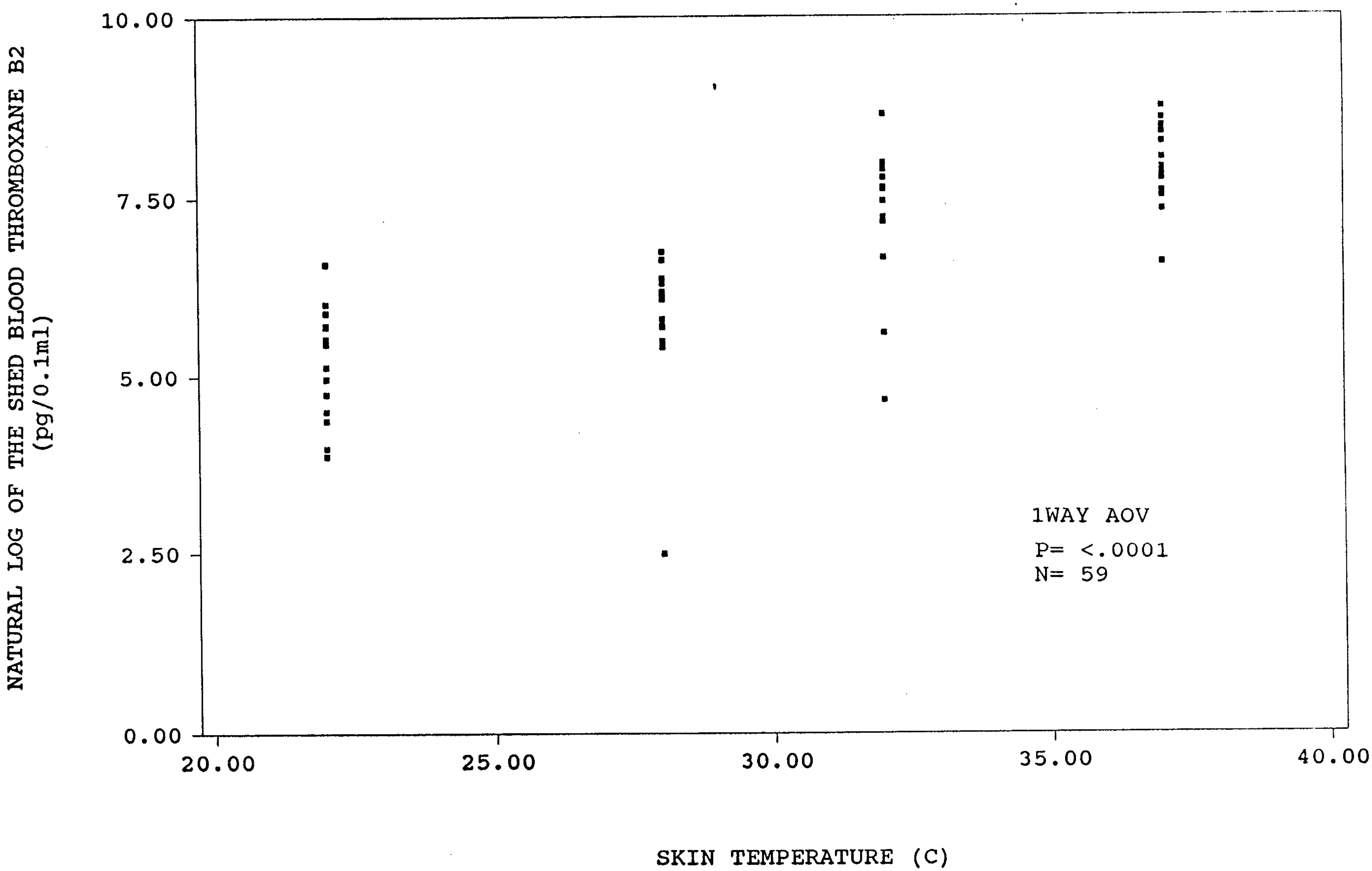

FIGURE 11

THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE PLASMA THROMBOXANE B_2 LEVEL AND THE SKIN TEMPERATURE

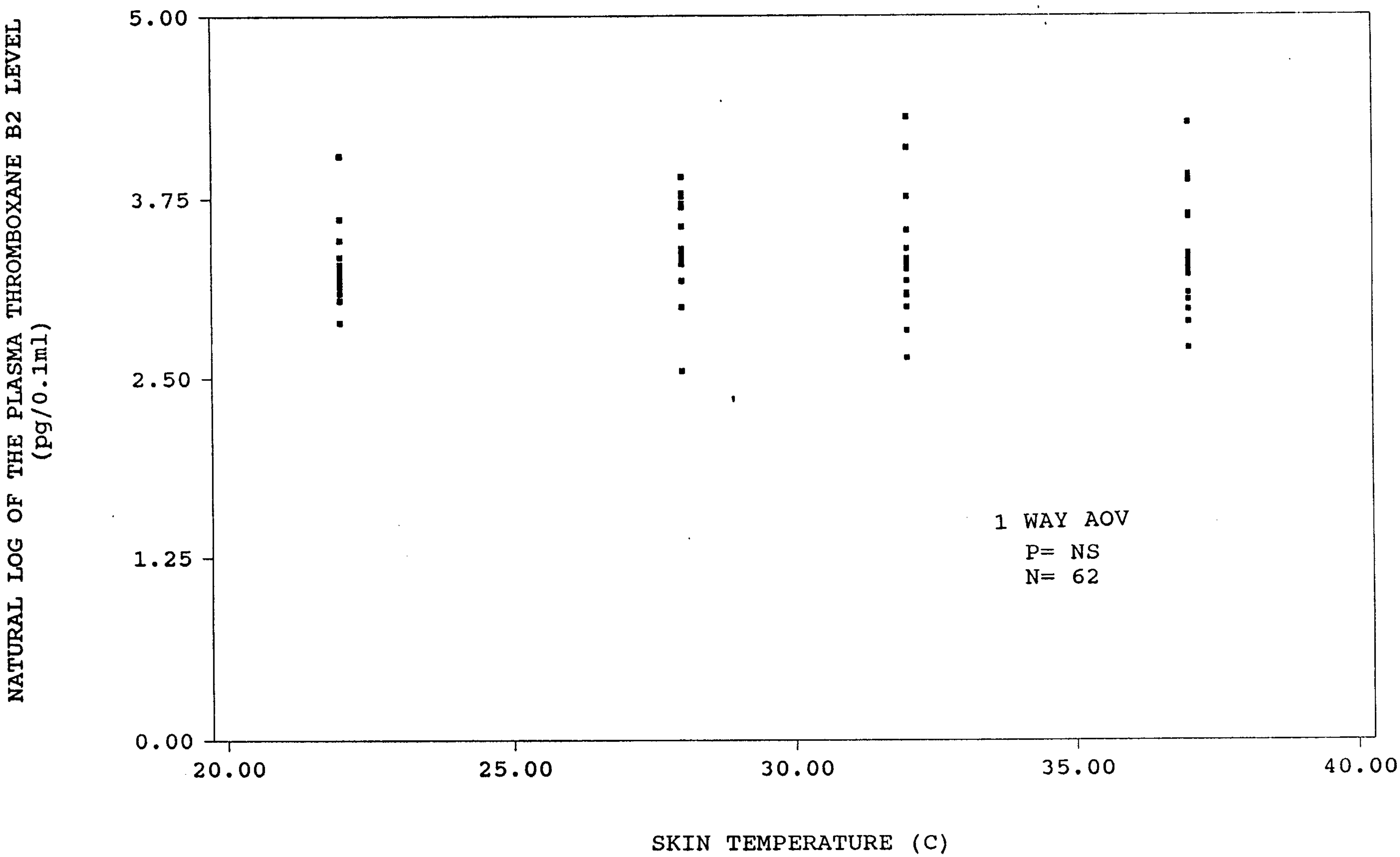

FIGURE 12

THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE SERUM THROMBOXANE B_2 LEVEL AND THE SKIN TEMPERATURE

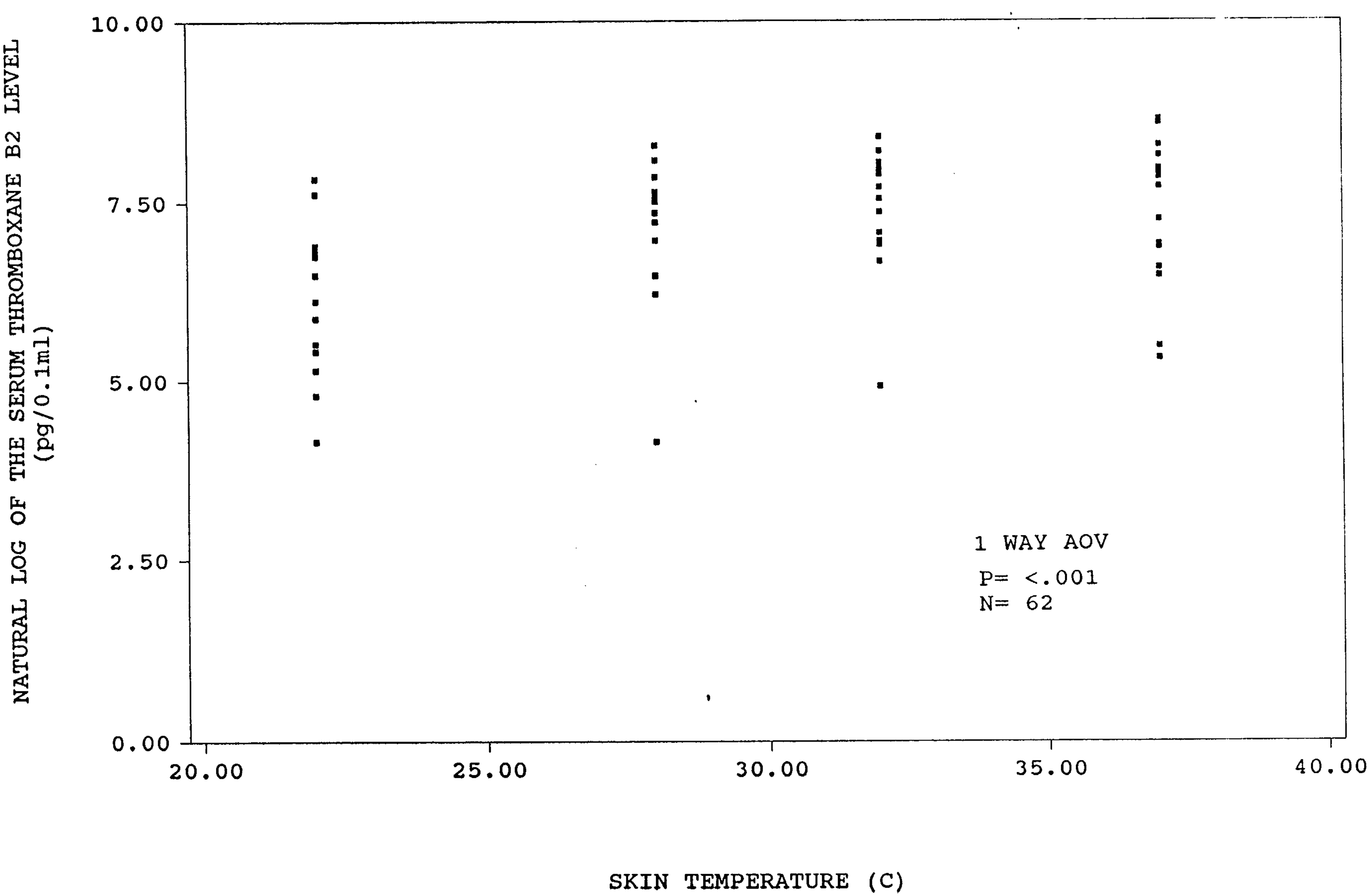

FIGURE 13

DISTRIBUTION OF THE NATURAL LOGARITHM OF THE BLEEDING TIME IN NORMAL VOLUNTEERS

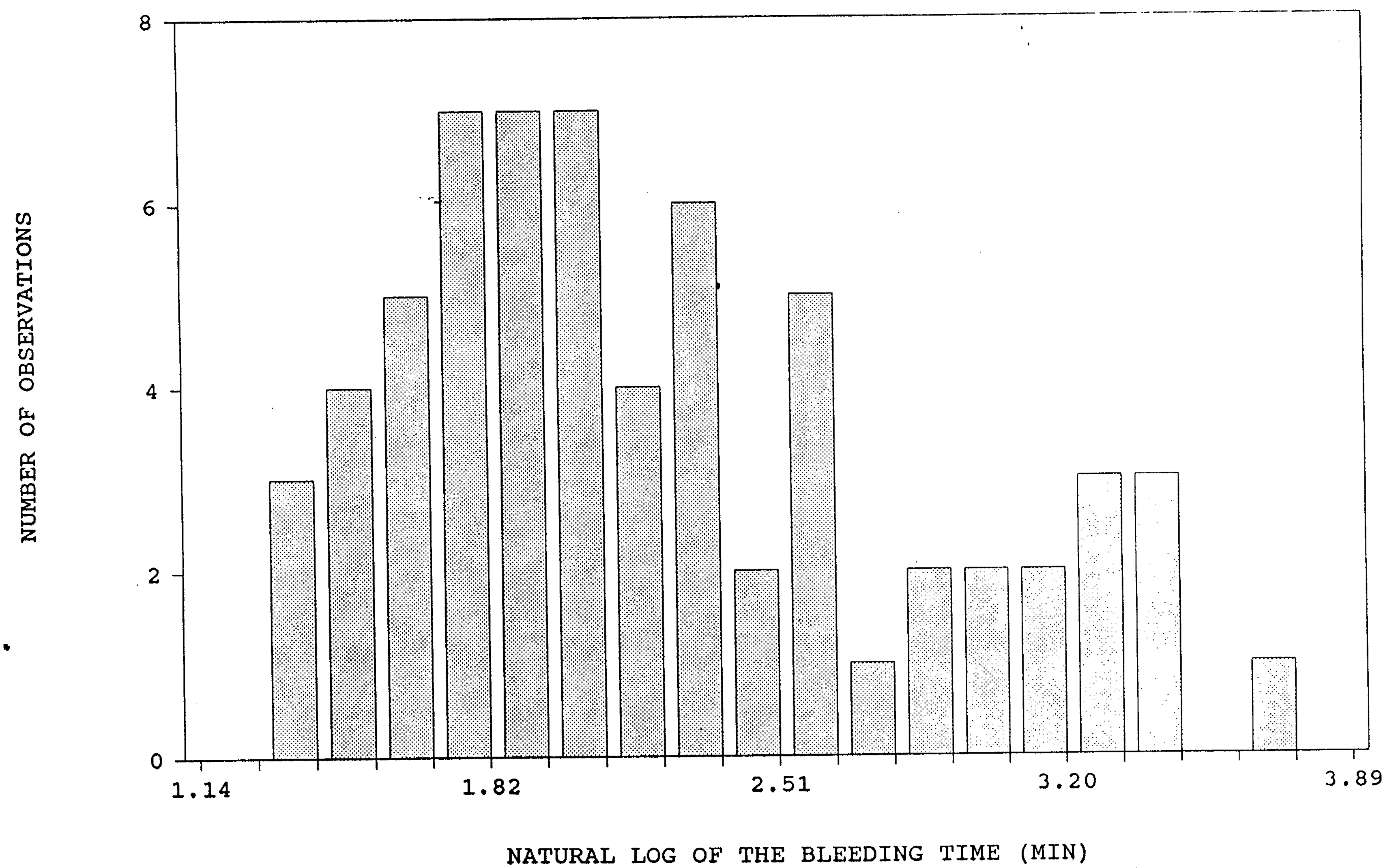

FIGURE 14

DISTRIBUTION OF THE NATURAL LOGARITHM OF THE SHED BLOOD THROMBOXANE B_2 LEVEL IN NORMAL VOLUNTEERS

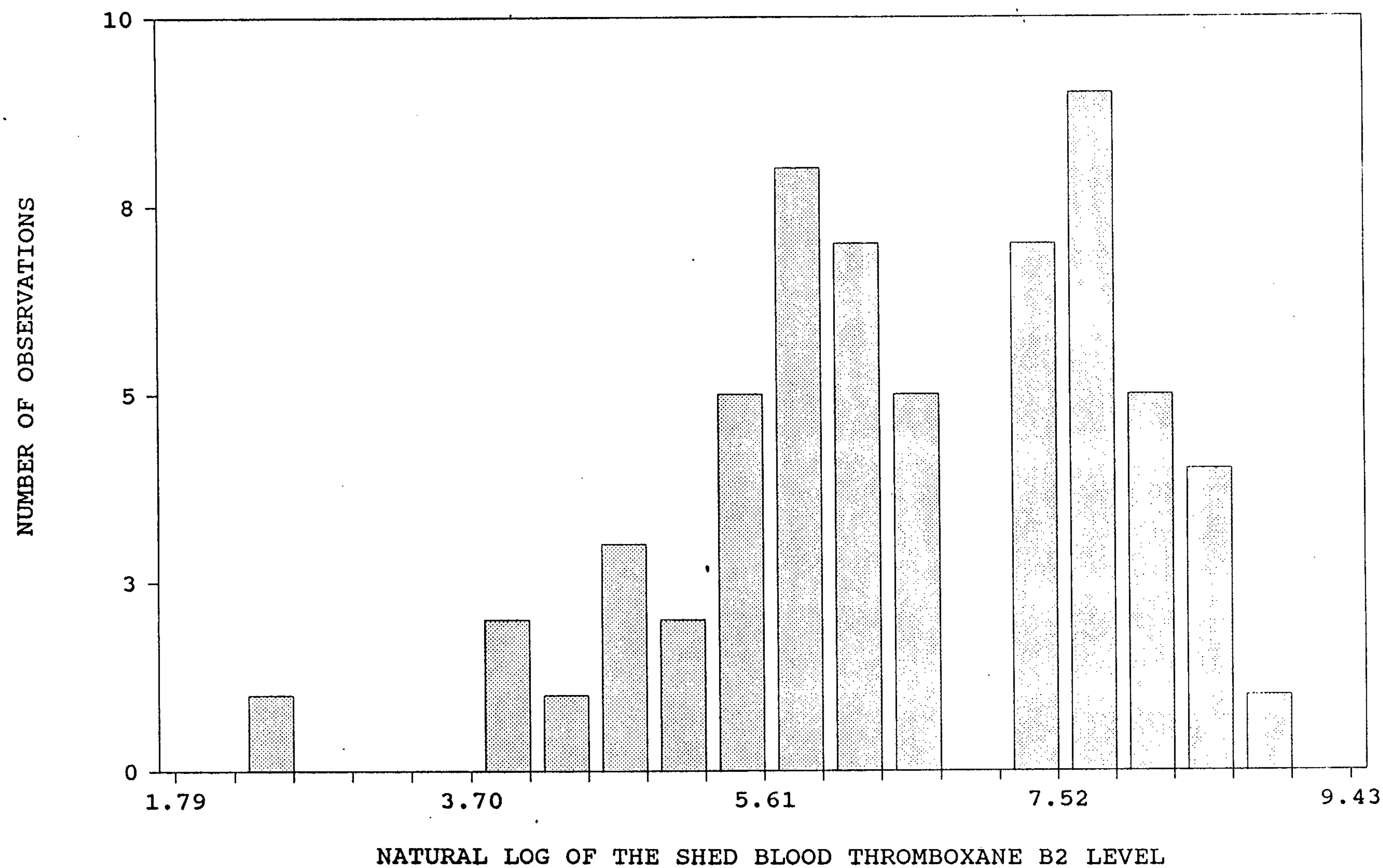

FIGURE 15

DISTRIBUTION OF THE NATURAL LOGARITHM OF THE PLASMA THROMBOXANE B_2 LEVEL IN NORMAL VOLUNTEERS

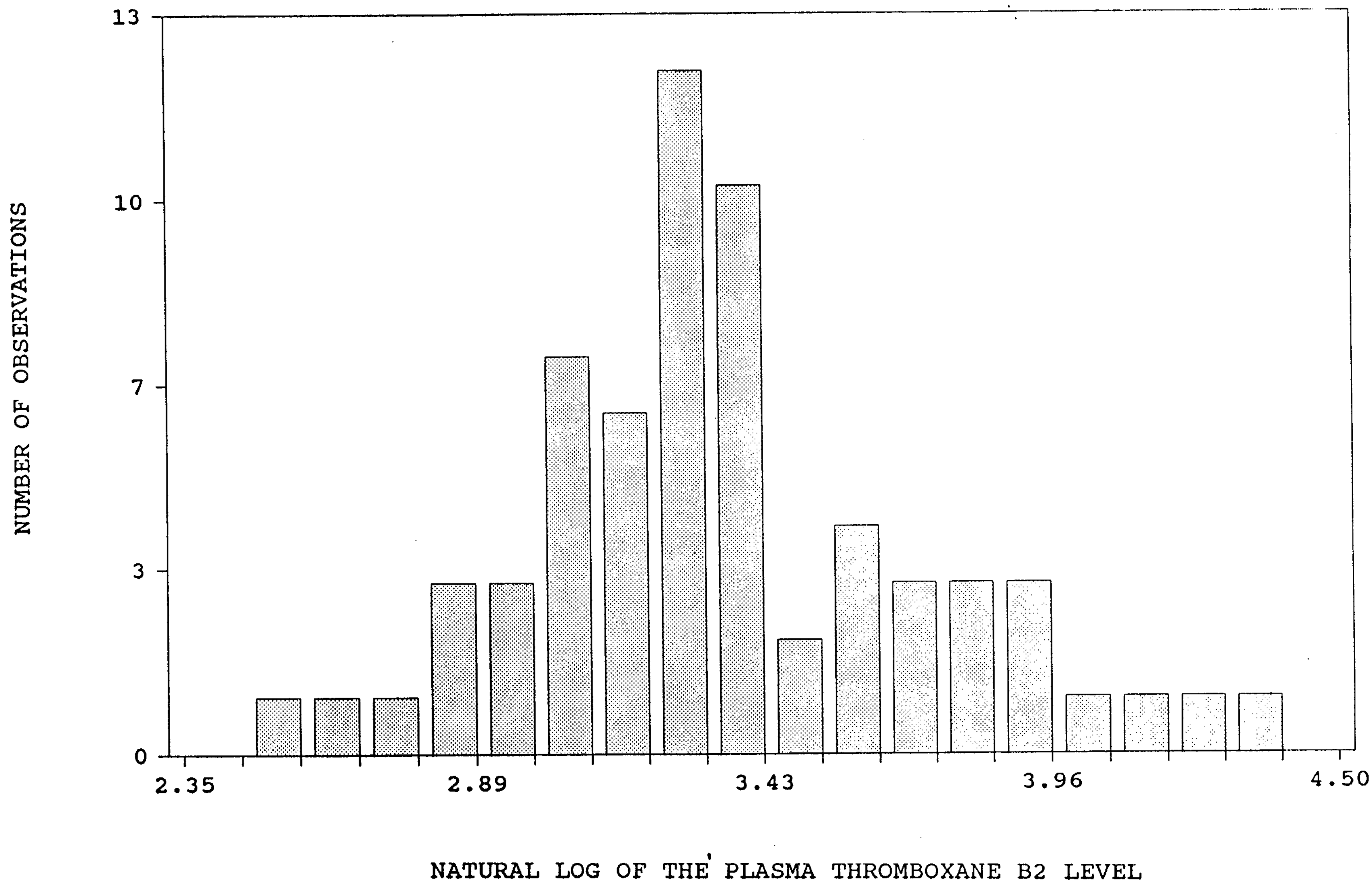

FIGURE 16

DISTRIBUTION OF THE NATURAL LOGARITHM OF THE SERUM THROMBOXANE B_2 LEVEL IN NORMAL VOLUNTEERS

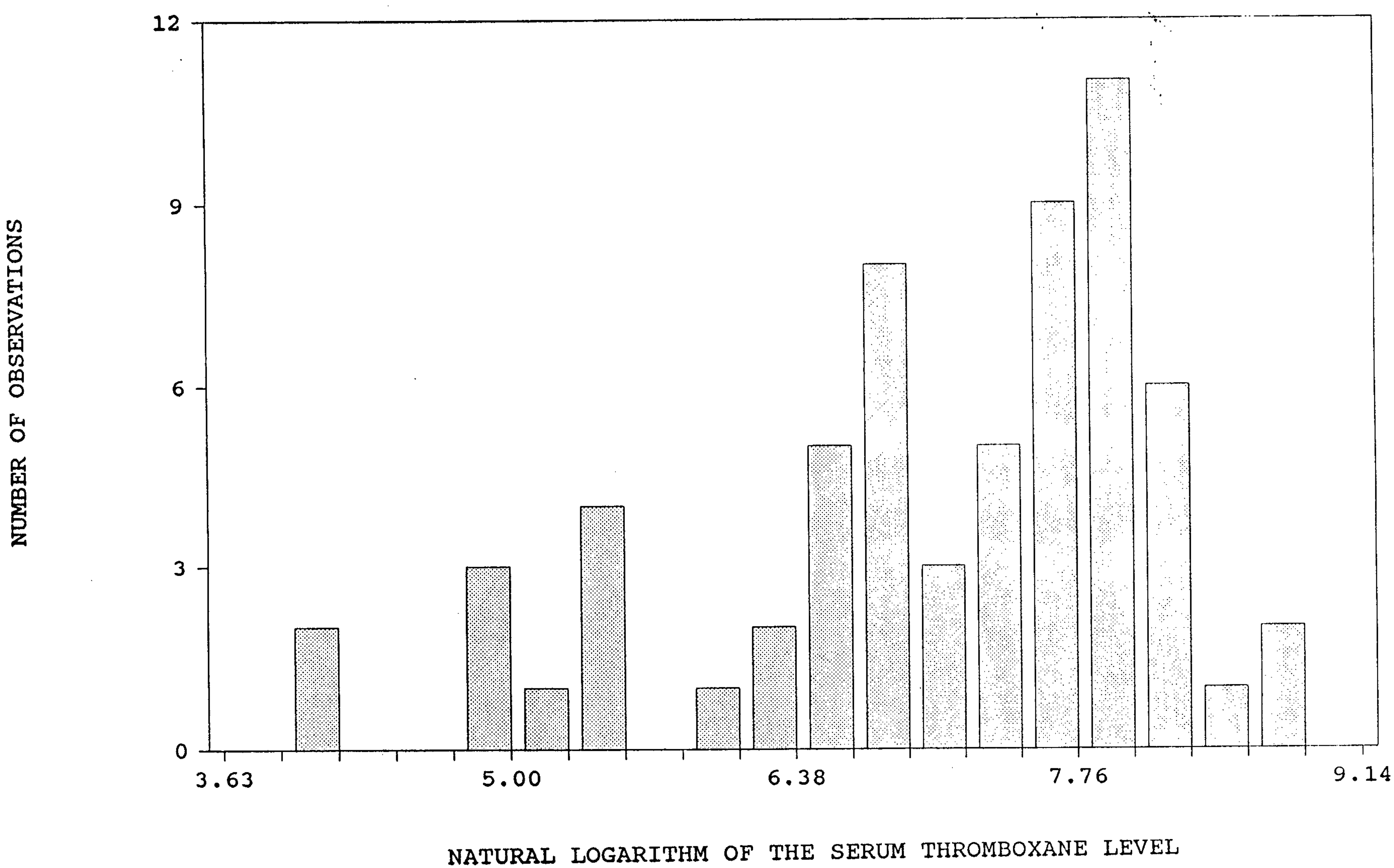

TABLE 4A

THE THROMBOXANE B_2 LEVEL IN A 0.6 ML VOLUME OF SHED BLOOD; IN ALL THE SHED BLOOD FROM ONE BLEEDING SITE AND IN TWO MINUTE COLLECTION INTERVALS AT ONE BLEEDING TIME SITE AND THE LEVEL IN THE FIRST 2 MINUTE INTERVAL AND THE LAST 2 MINUTE INTERVAL REPORTED TOGETHER WITH THE BLEEDING TIME MEASURED AT THE LOCAL SKIN TEMPERATURE OF +32C

	Collection to 0.6 ml volume of shed blood pg/0.1 ml	Collection of all the shed blood from 1 site pg/0.1 ml	2 Minute Collection intervals from 1 site Intervals First 2 min pg/0.1 ml	Last 2 min pg/0.1 ml	Mean BT (min) at 32C
Mean:	632	1345	405	1534	8.0
SD:	318	745	220	747	3.5
Range:	278-1134	425-2588	110-751	570-2415	6.5-14
n:	9	9	9	9	9

Paired T test between	
the first two minutes and the last two minute sample	<.05

FIGURE 17

THE SHED BLOOD THROMBOXANE B_2 LEVEL MEASURED DURING THE BLEEDING TIME AT TWO MINUTE INTERVALS

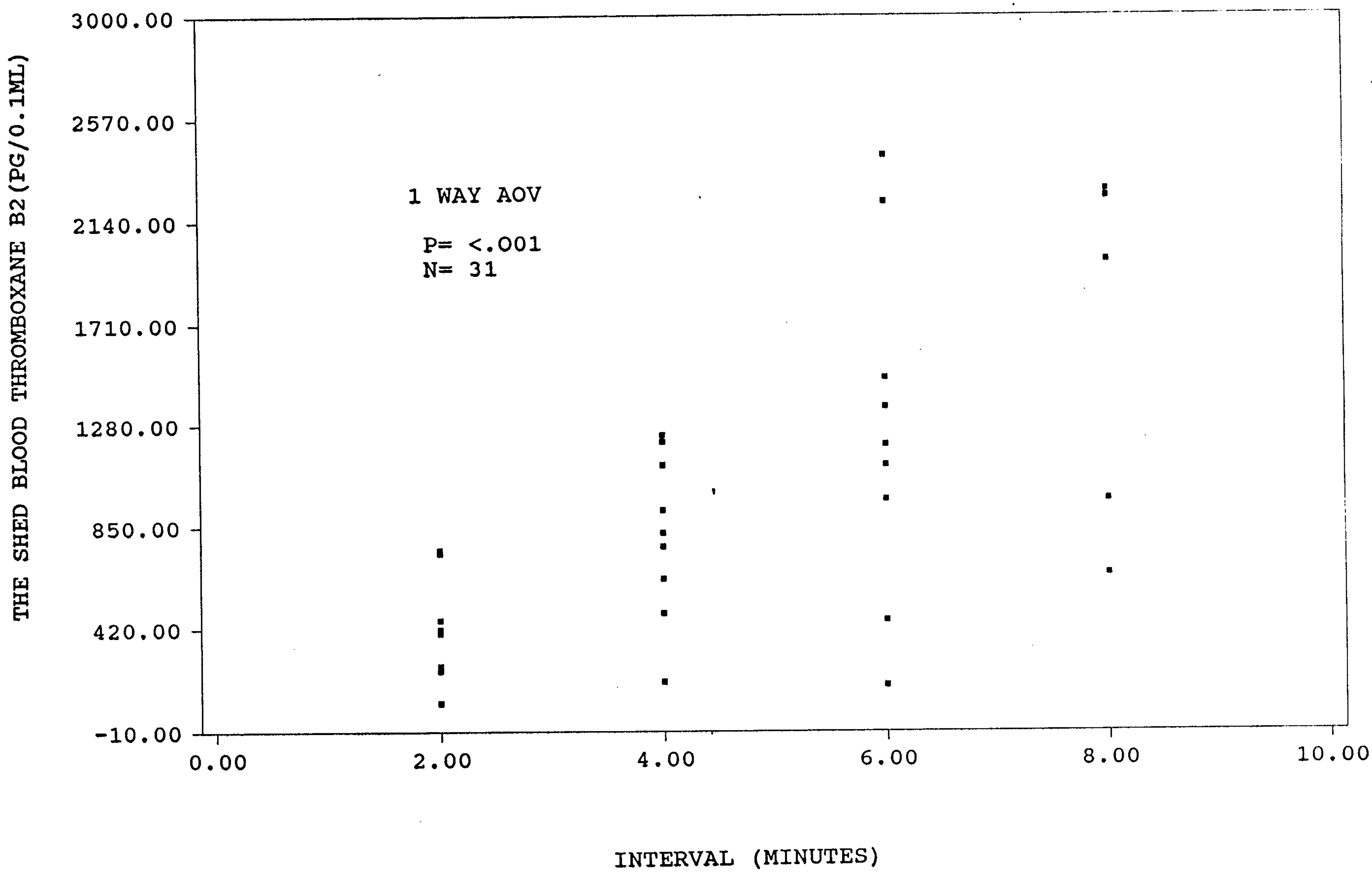

FIGURE 18

THE THROMBOXANE B_2 LEVEL IN SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

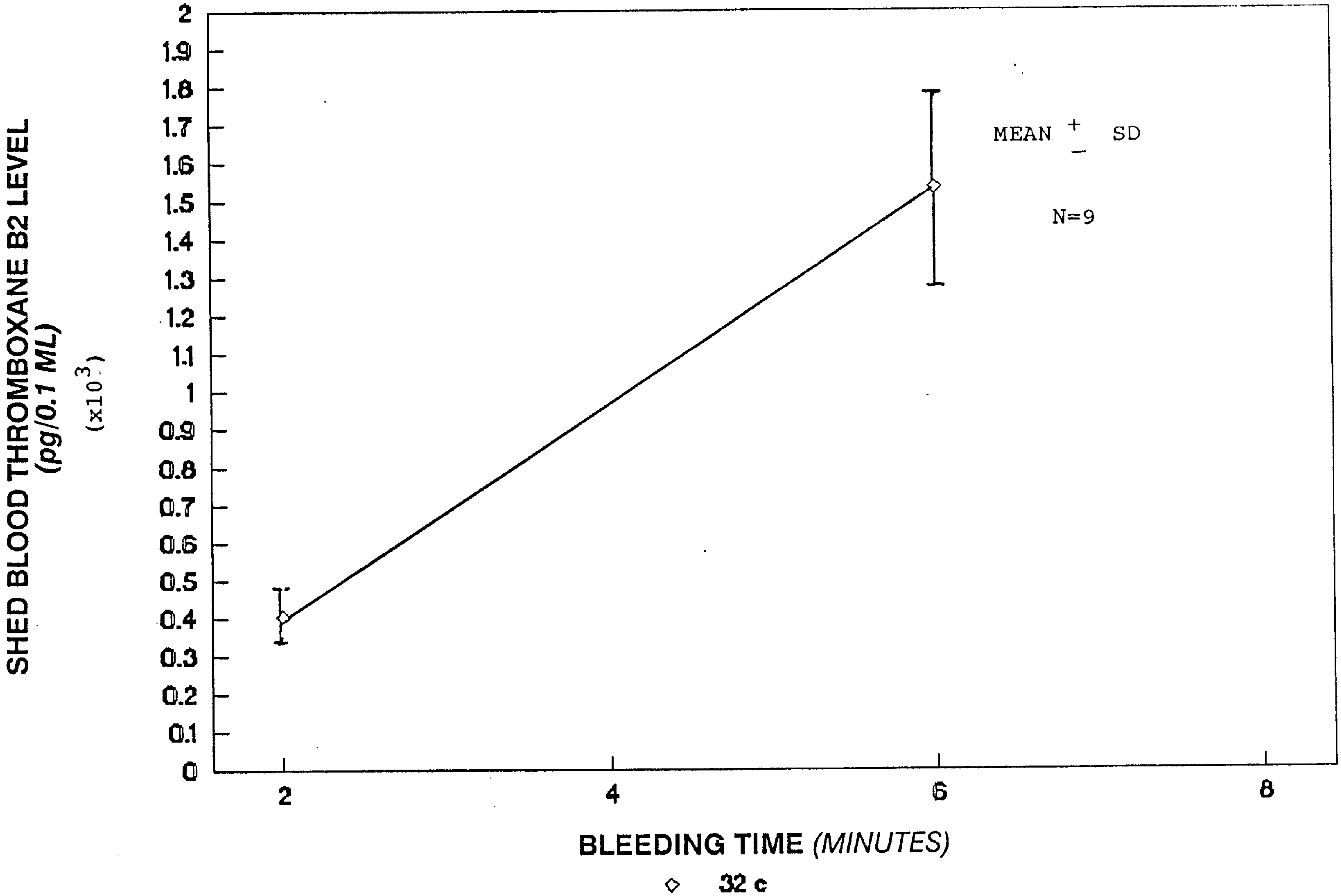

FIGURE 19

THE THROMBOXANE B_2 LEVEL IN THE SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

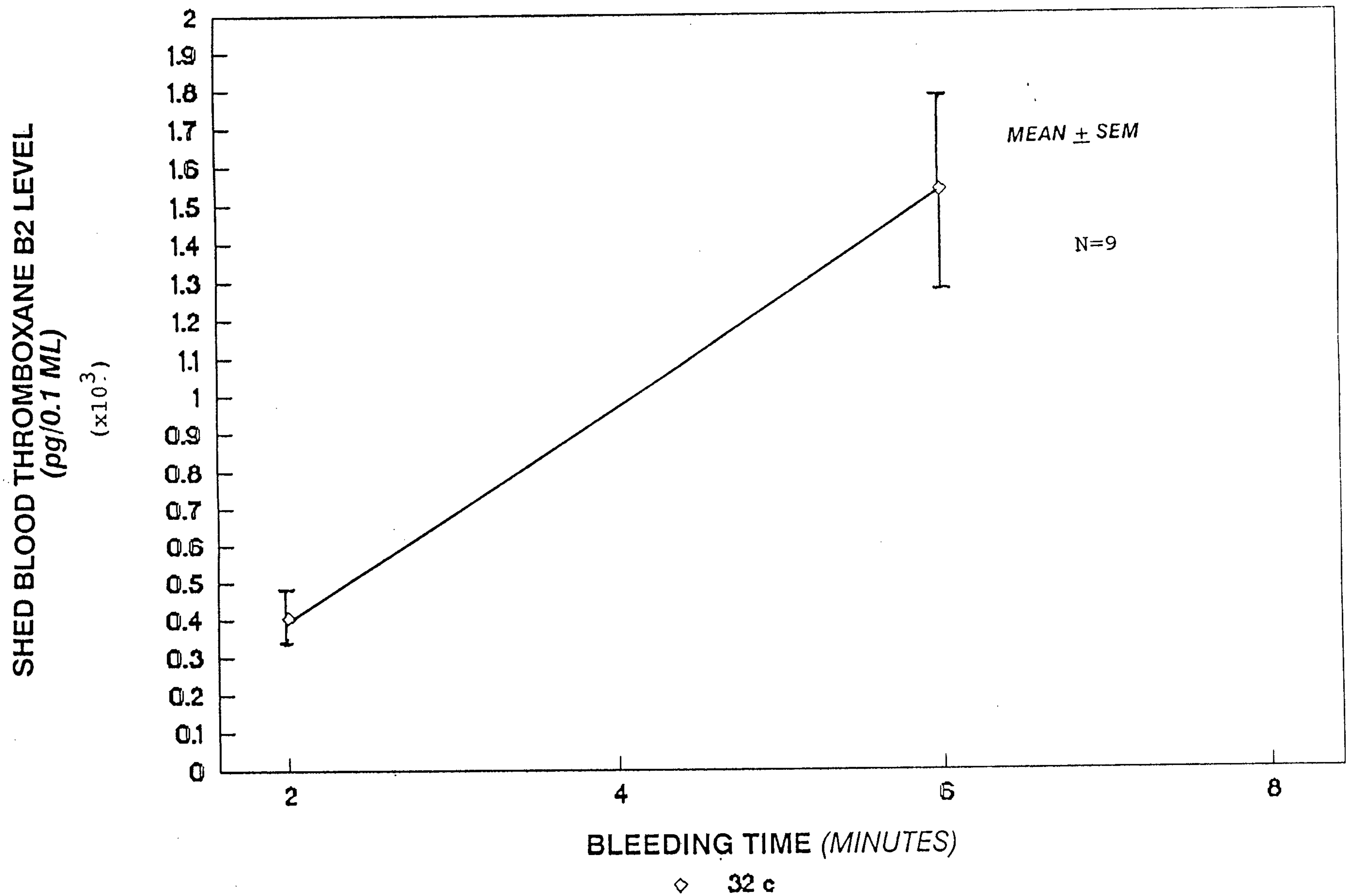

TABLE 4B

THE NATURAL LOGARITHM OF THE THROMBOXANE B_2 LEVEL IN A 0.6 ML VOLUME OF SHED BLOOD; IN ALL THE SHED BLOOD FROM ONE BLEEDING SITE AND IN TWO MINUTE COLLECTION INTERVALS AT ONE BLEEDING TIME SITE AND THE LEVEL IN THE FIRST 2 MINUTE INTERVAL AND THE LAST 2 MINUTE INTERVAL REPORTED TOGETHER WITH THE BLEEDING TIME MEASURED AT THE LOCAL SKIN TEMPERATURE OF +32C

	Collection to 0.6 ml volume of shed blood pg/0.1 ml	Collection of all the shed blood from 1 site pg/0.1 ml	2 Minute Collection intervals from 1 site Intervals		Mean BT (min) at 32C
			First 2 min pg/0.1 ml	Last 2 min pg/0.1 ml	
Mean:	6.333	7.010	5.857	7.185	2.047
SD:	.520	.704	.601	.643	.333
n:	9	9	9	9	9
Paired T test between the first 2 minute and the last two minute sample			<.001		

FIGURE 20

THE NATURAL LOGARITHM OF THE SHED BLOOD THROMBOXANE B_2 LEVEL MEASURED DURING THE BLEEDING TIME AT TWO MINUTE INTERVALS

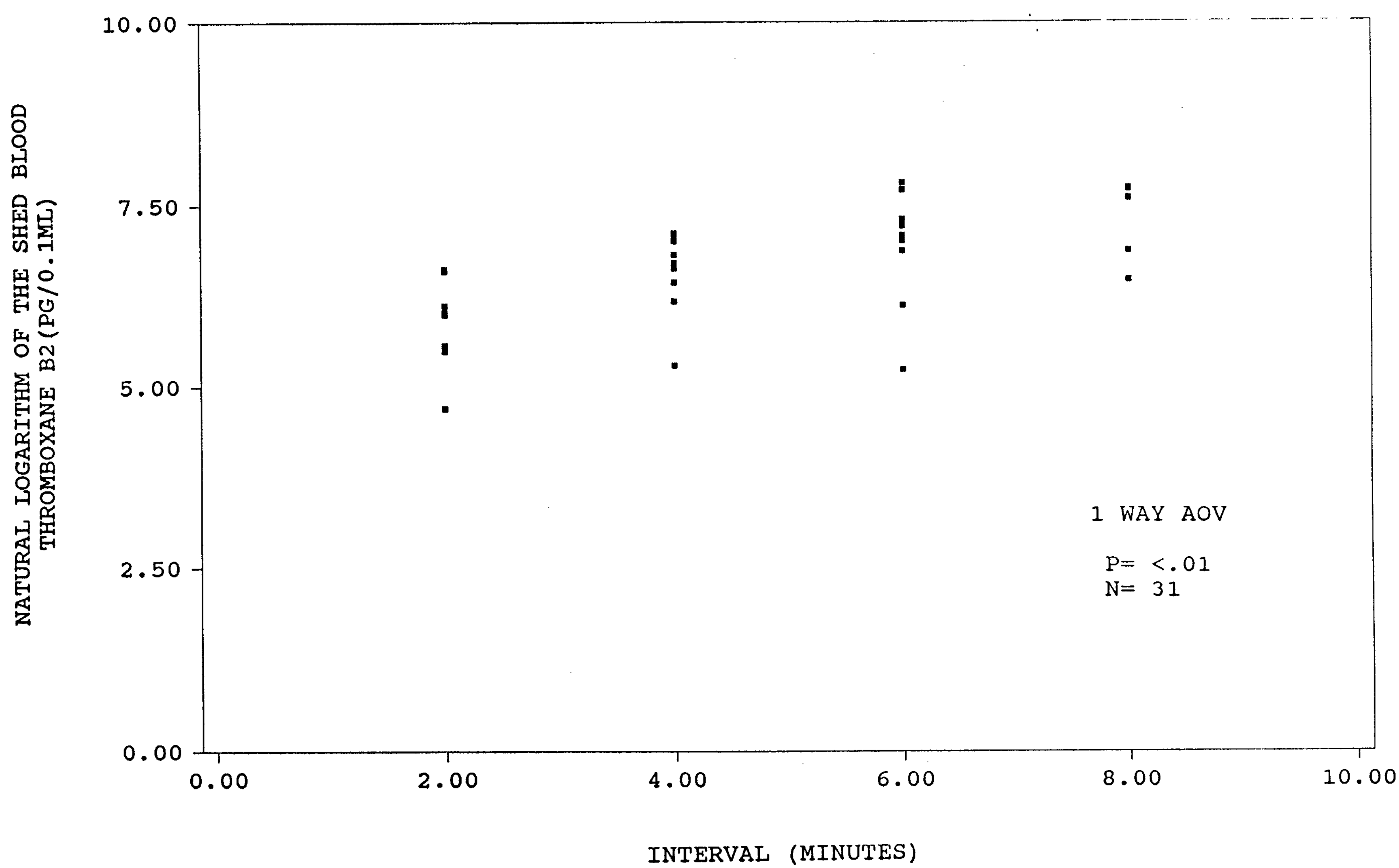

FIGURE 21

THE NATURAL LOGARITHM OF THE THROMBOXANE B_2 LEVEL IN SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

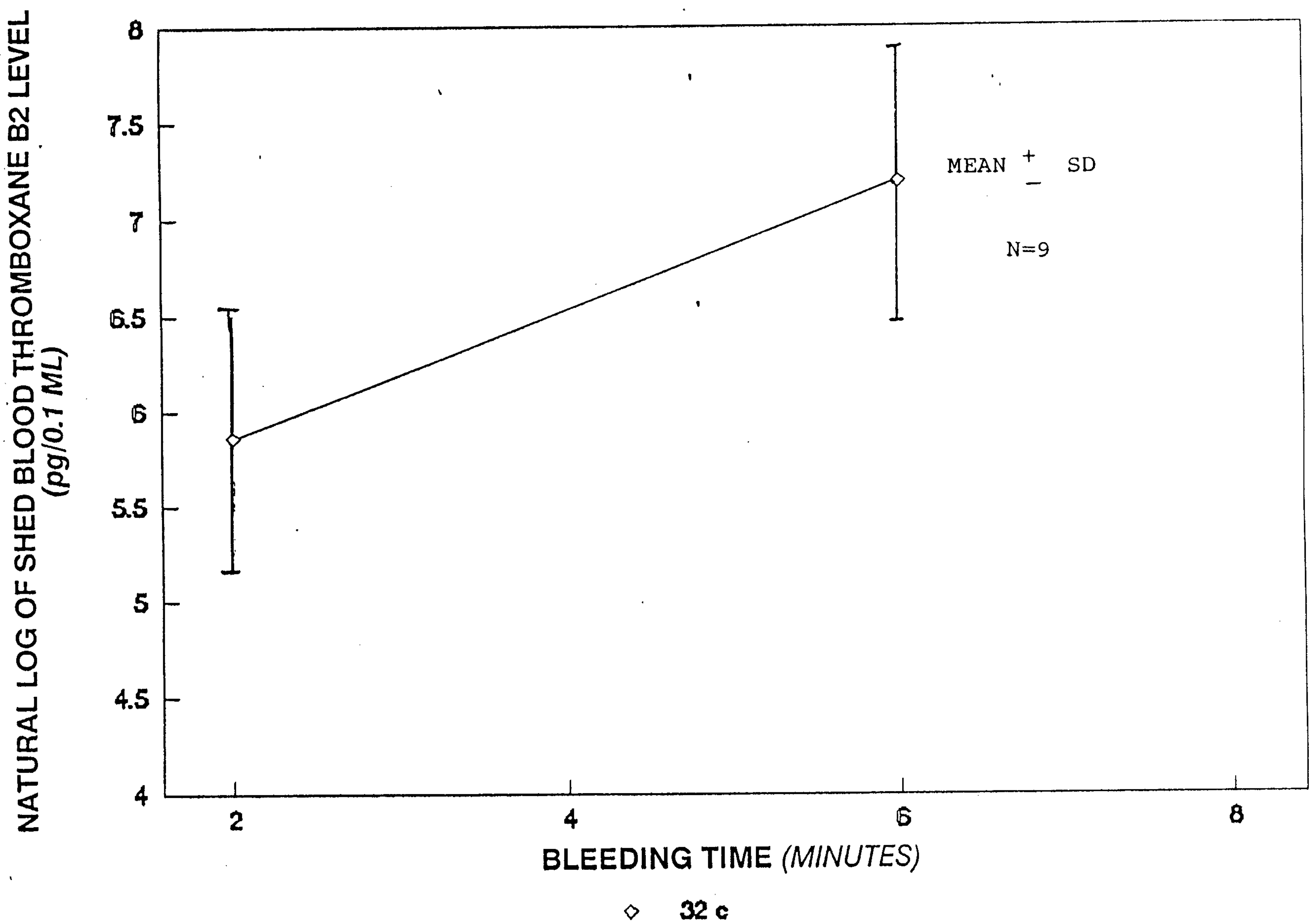

FIGURE 22

THE NATURAL LOGARITHM OF THE THROMBOXANE B_2 LEVEL IN SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

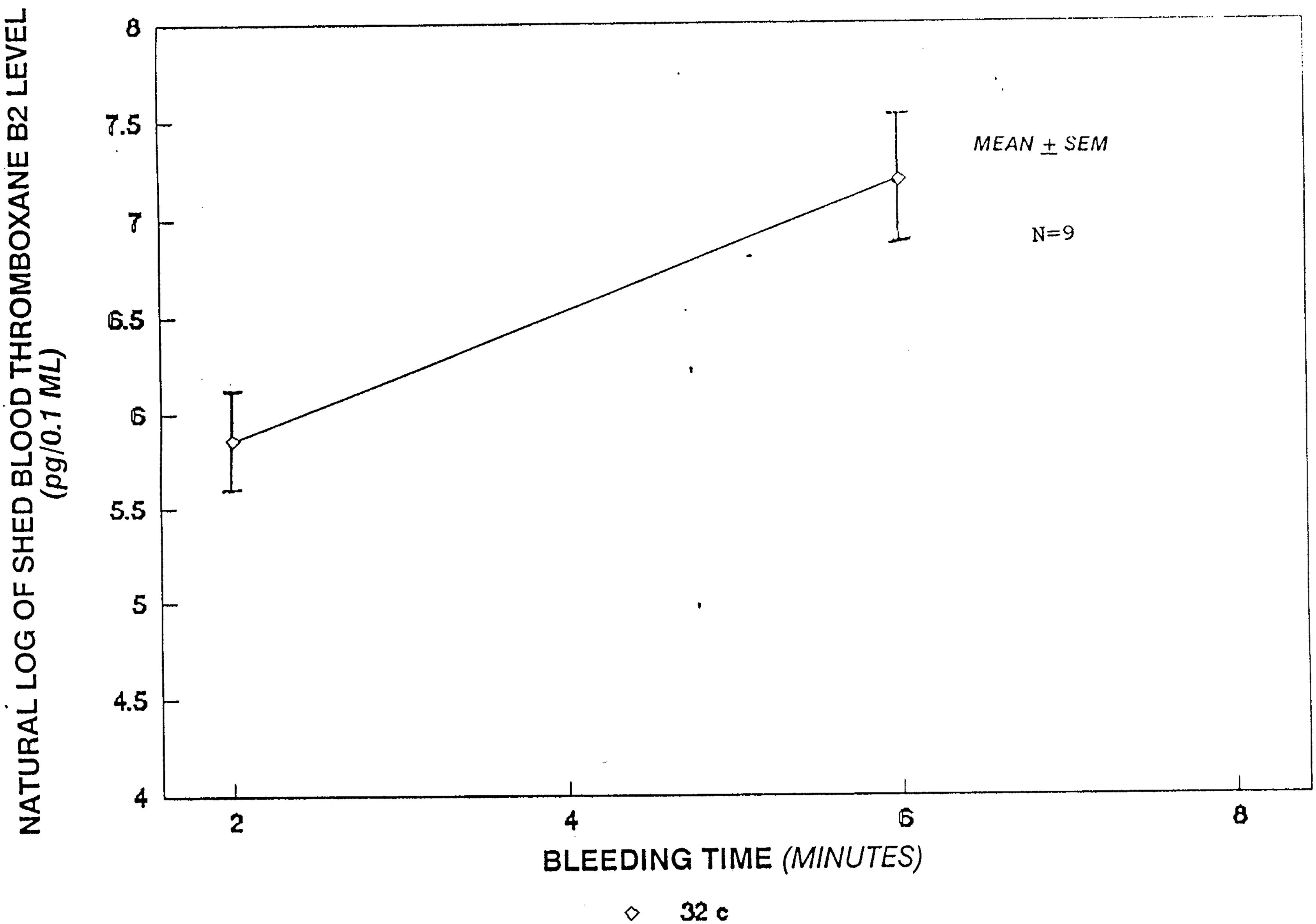

TABLE 5A

SHED BLOOD TXB2 MEASUREMENTS IN BLOOD COLLECTED FROM A BLEEDING TIME SITE USING THREE METHODS OF COLLECTION AT THE LOCAL SKIN TEMPERATURES OF +32C, +28C, AND +22C

	Collection of 0.6 ml shed blood (pg/0.1 ml)	Collected of all shed blood from BT site (pg/0.1 ml)	Shed blood collected for first 2 min from 1 site (pg/0.1 ml)		Shed blood collected for last 2 min from 1 site (pg/0.1 ml)	BT in Min
32C						
Mean:	801	919	416		1602	8.1
SD:	436	527	326		1165	2.4
n:	6	6	6		6	6
				=.05*		
28C						
Mean:	572	439	339		985	11.2
SD:	472	217	177		595	3.1
n:	6	6	6		6	6
				<.05*		
22C						
Mean:	72	104	228		129	23
SD:	35	92	194		51	6
n:	5	5	5		5	5
				NS		

* Paired T-test between first 2 minute collection and the last 2 minute collection.

FIGURE 23

THE SHED BLOOD THROMBOXANE B_2 LEVEL MEASURED AT TWO MINUTE INTERVALS DURING THE BLEEDING TIME WHEN THE SKIN TEMPERATURE WAS +32C

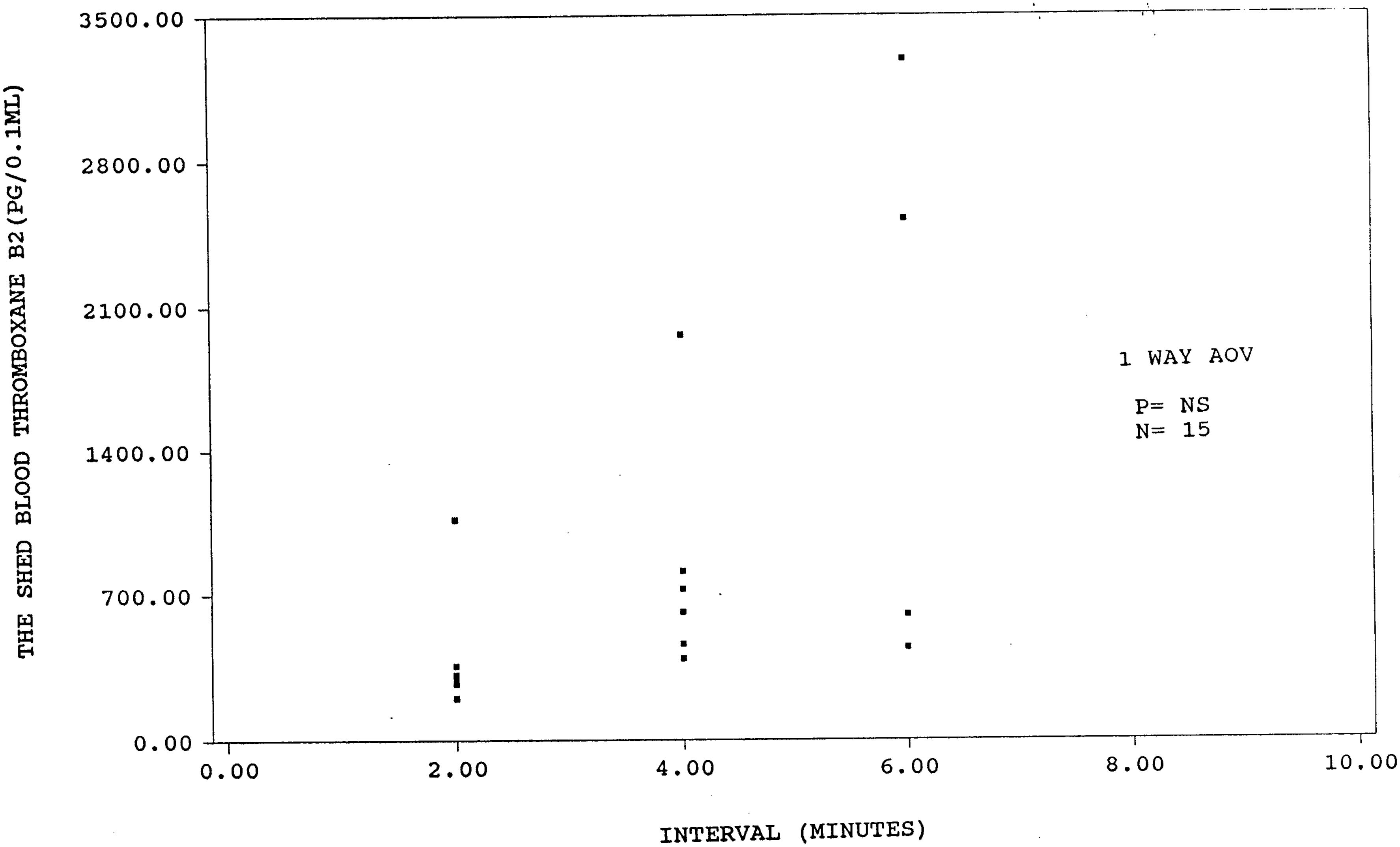

FIGURE 24

THE SHED BLOOD THROMBOXANE B_2 LEVEL MEASURED AT TWO MINUTE INTERVALS DURING THE BLEEDING TIME WHEN THE SKIN TEMPERATURE WAS +28C

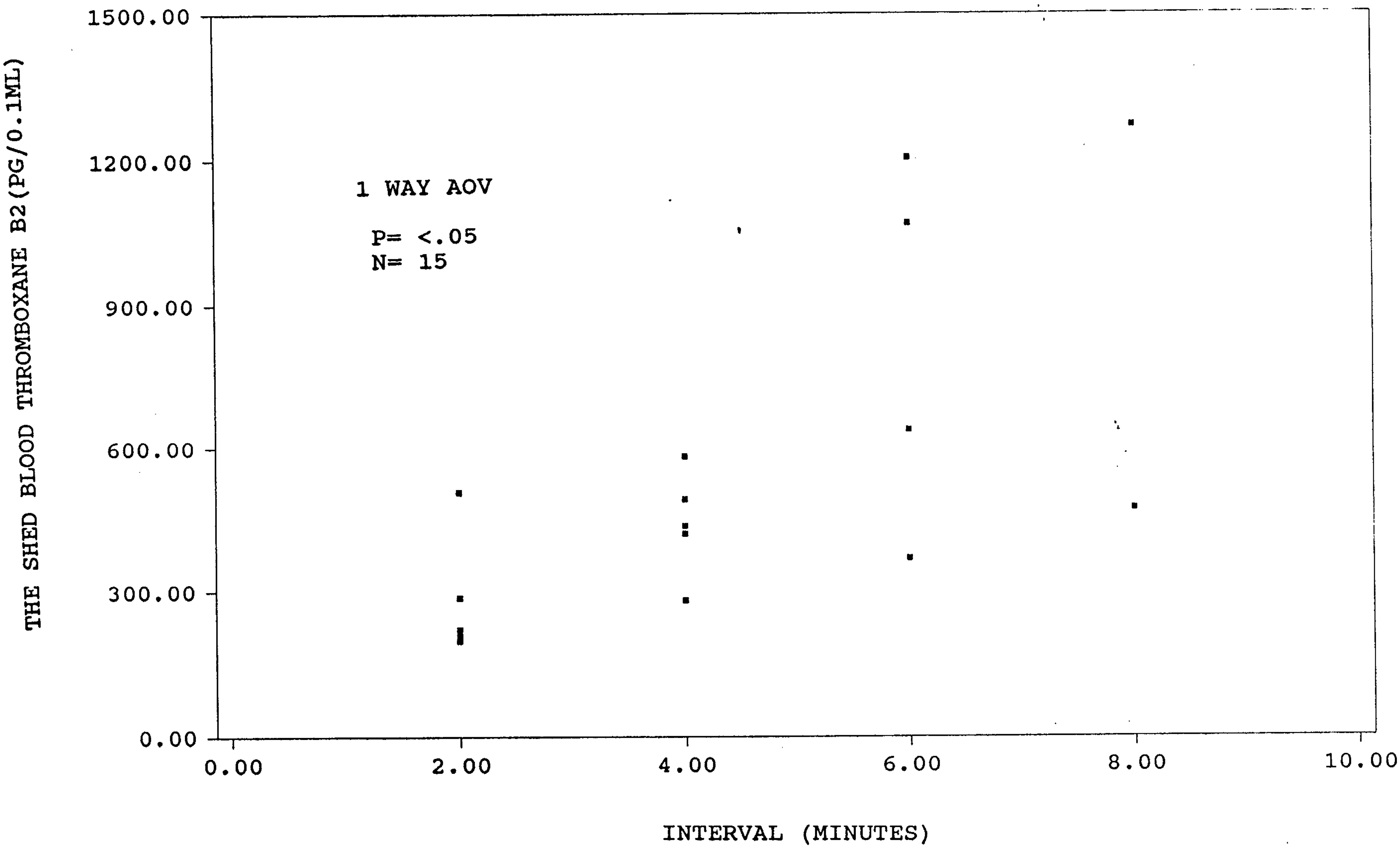

FIGURE 25

THE SHED BLOOD THROMBOXANE B_2 LEVEL MEASURED AT TWO MINUTE INTERVALS DURING THE BLEEDING TIME WHEN THE SKIN TEMPERATURE WAS +22C

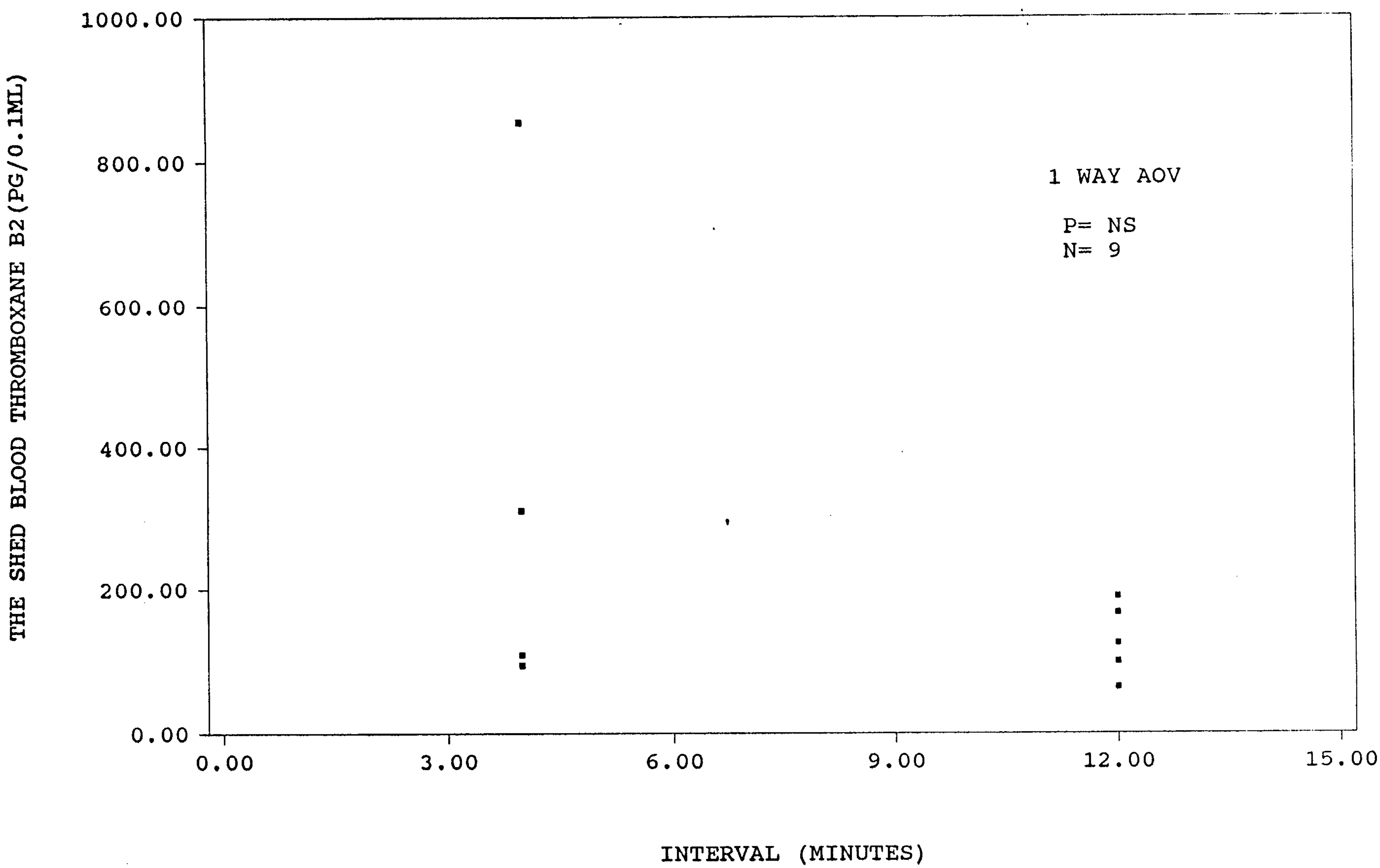

FIGURE 26

THE THROMBOXANE B_2 LEVEL IN SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

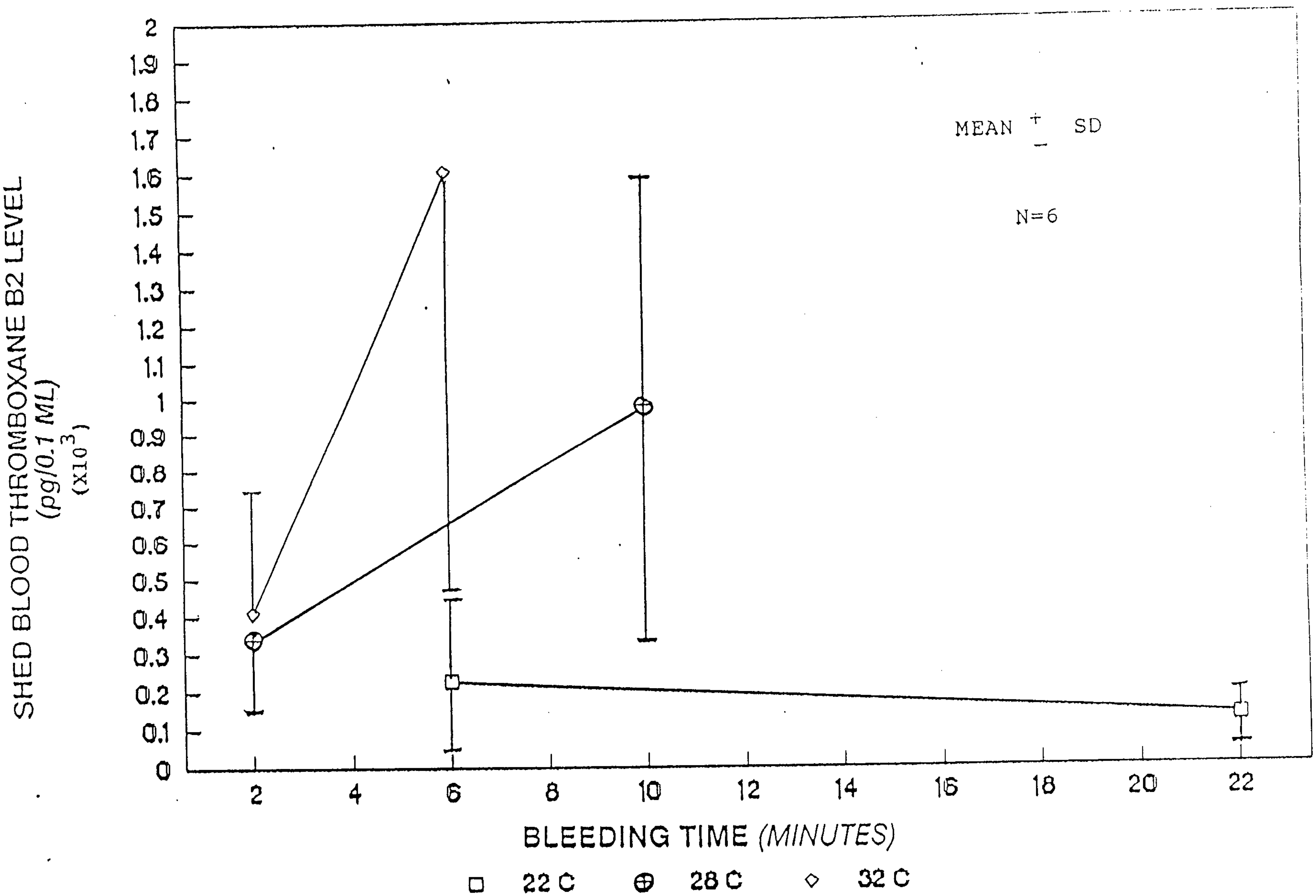

FIGURE 27

THE THROMBOXANE B_2 LEVEL IN SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

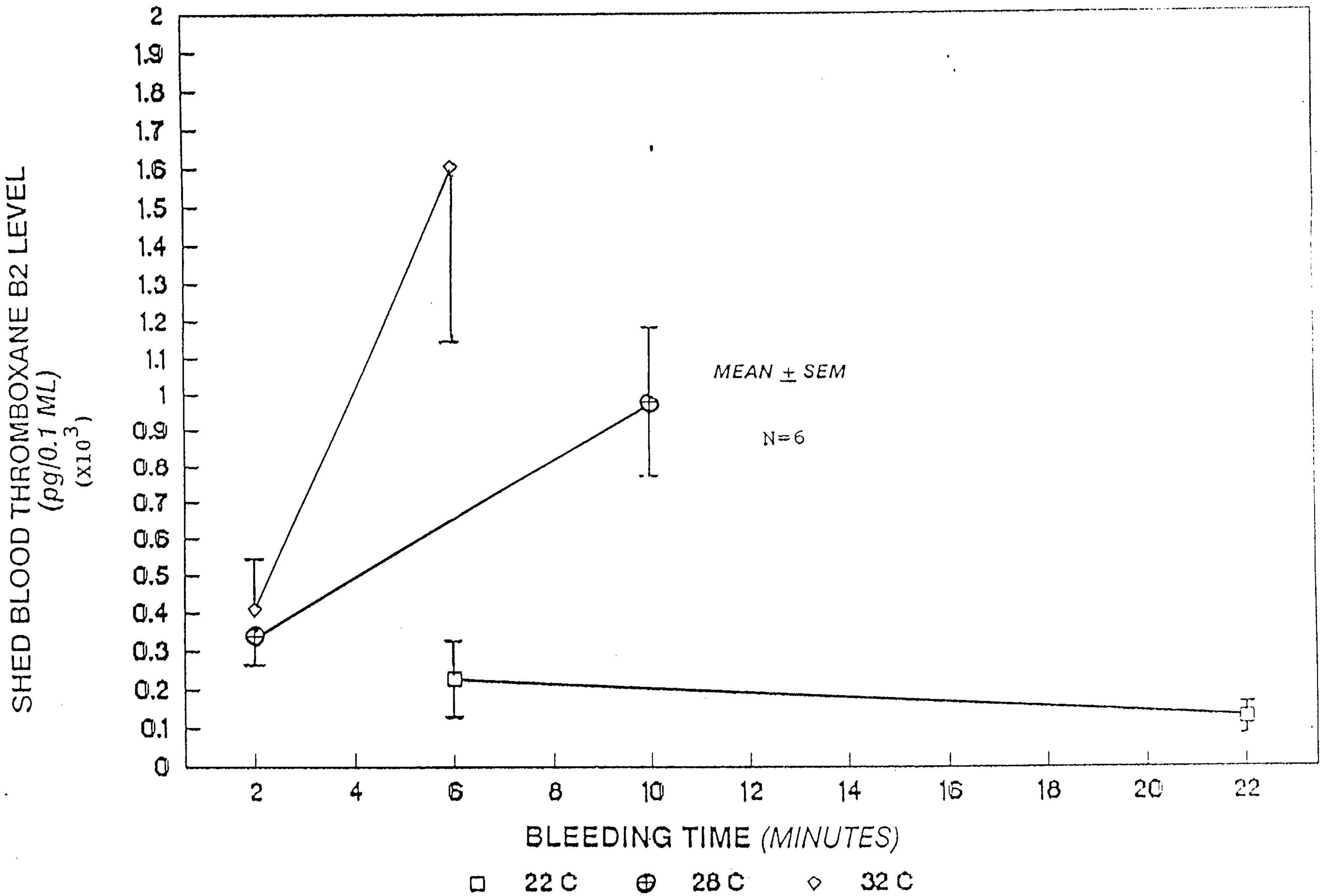

TABLE 5B

THE NATURAL LOGARITHM OF THE SHED BLOOD TXB2 MEASUREMENTS IN BLOOD COLLECTED FROM A BLEEDING TIME SITE USING THREE METHODS OF COLLECTION AT THE LOCAL SKIN TEMPERATURES OF +32C, +28C, AND +22C

N=6	Collection of 0.6 ml shed blood (pg/0.1 ml)	Collected of all shed blood from BT site (pg/0.1 ml)	Shed blood collected for first 2 min from 1 site (pg/0.1 ml)	Shed blood collected for last 2 min from 1 site (pg/0.1 ml)	BT in Min
32C					
Mean:	6.543	6.699	5.863	7.115	2.047
SD:	.610	.537	.575	.830	.333
n:	6	6	6	6	6
Paired T*:			<.05*		
28C					
Mean:	6.114	5.66	5.722	6.480	2.374
SD:	.72	.25	.487	.713	.302
n:	6	6	6	6	6
Paired T*:			<.05*		
22C					
Mean:	4.18	4.5	5.2	4.78	3.13
SD:	.47	.7	.99	.4	.3
n:	5	5	5	5	5
			NS*		

* Paired T-test between first 2 minute collection and the last 2 minute collection.

FIGURE 28

THE NATURAL LOGARITHM OF THE SHED BLOOD THROMBOXANE B_2 LEVEL MEASURED AT TWO MINUTE INTERVAL DURING THE BLEEDING TIME WHEN THE SKIN TEMPERATURE WAS +32C

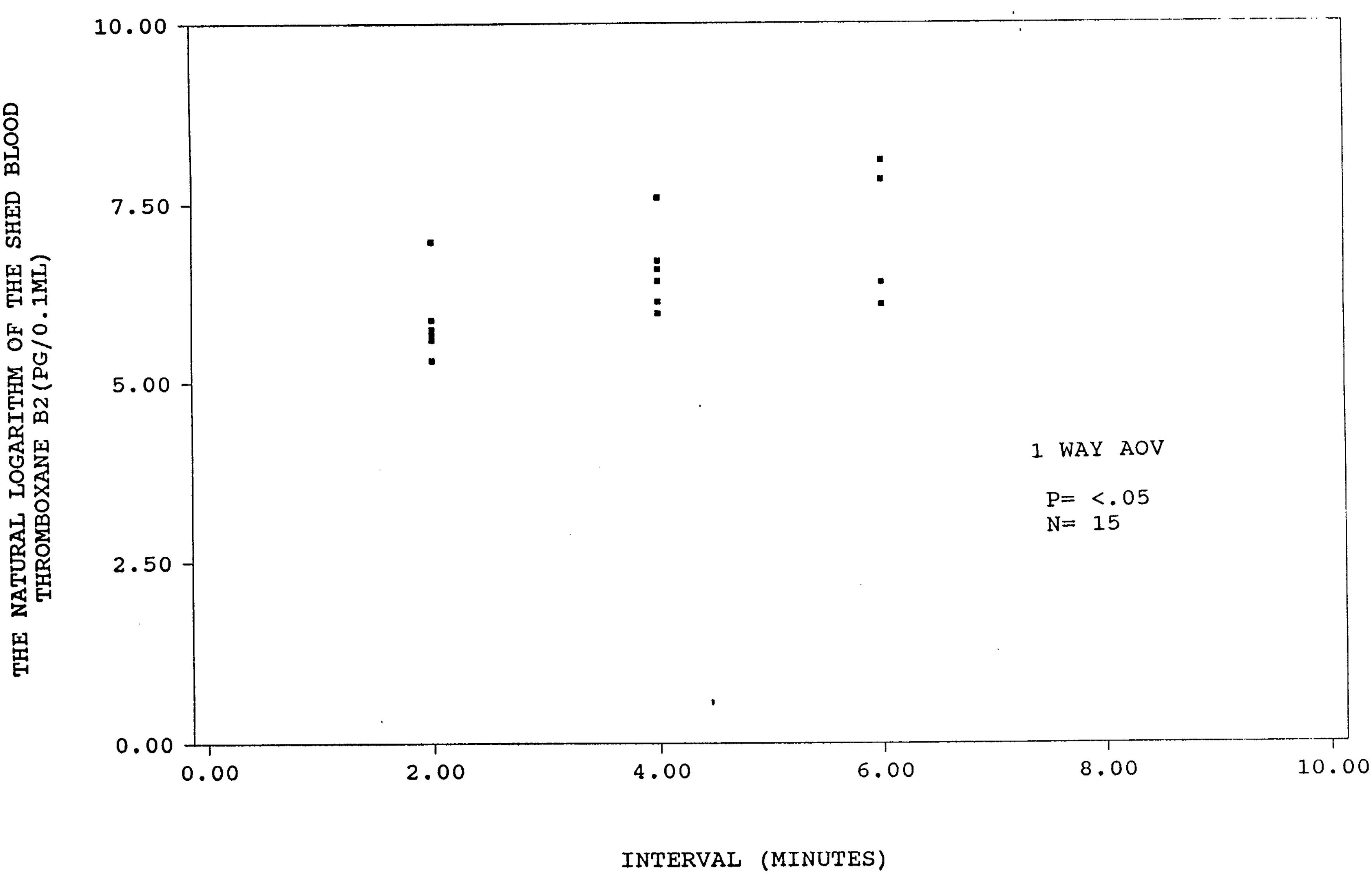

FIGURE 29

THE NATURAL LOGARITHM OF THE SHED BLOOD THROMBOXANE B_2 LEVEL MEASURED AT TWO MINUTE INTERVALS DURING THE BLEEDING TIME WHEN THE THE SKIN TEMPERATURE WAS +28C

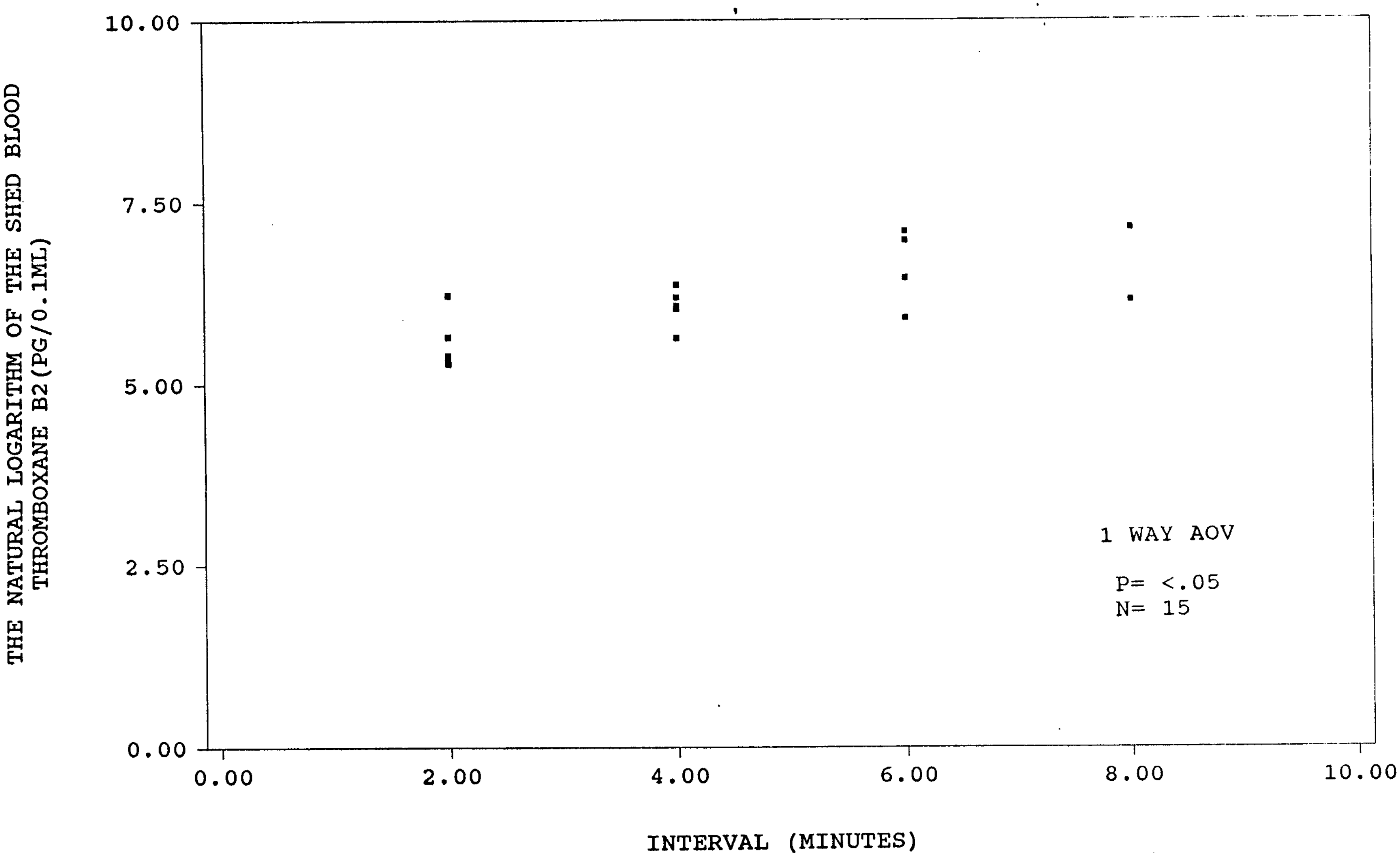

FIGURE 30

THE NATURAL LOGARITHM OF THE SHED BLOOD THROMBOXANE B_2 LEVEL MEASURED AT TWO MINUTE INTERVALS DURING THE BLEEDING TIME WHEN THE SKIN TEMPERATURE WAS +22C

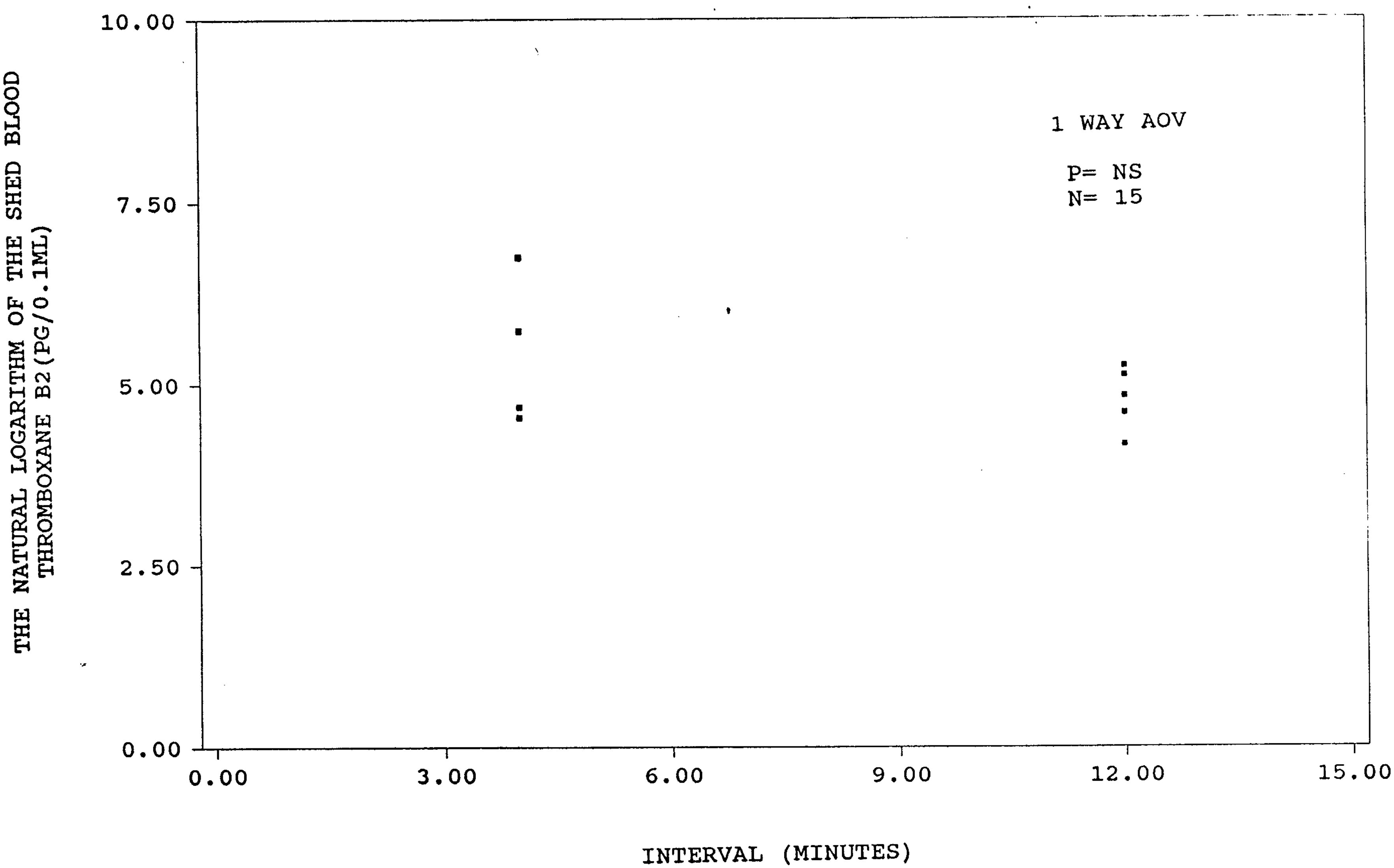

FIGURE 31

THE NATURAL LOGARITHM OF THE THROMBOXANE B_2 LEVEL IN SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

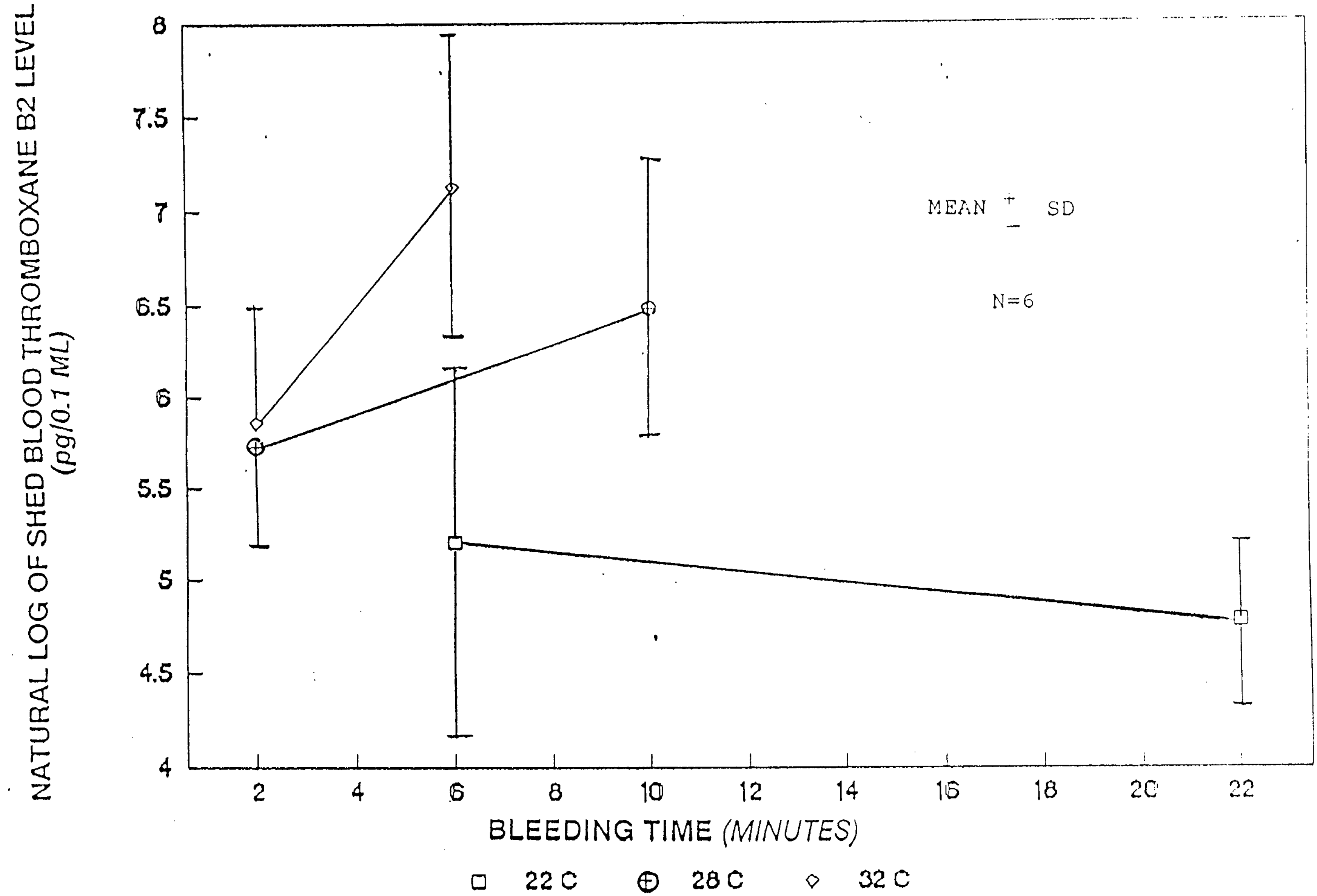

FIGURE 32

THE NATURAL LOGARITHM OF THE THROMBOXANE B_2 LEVEL IN THE SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

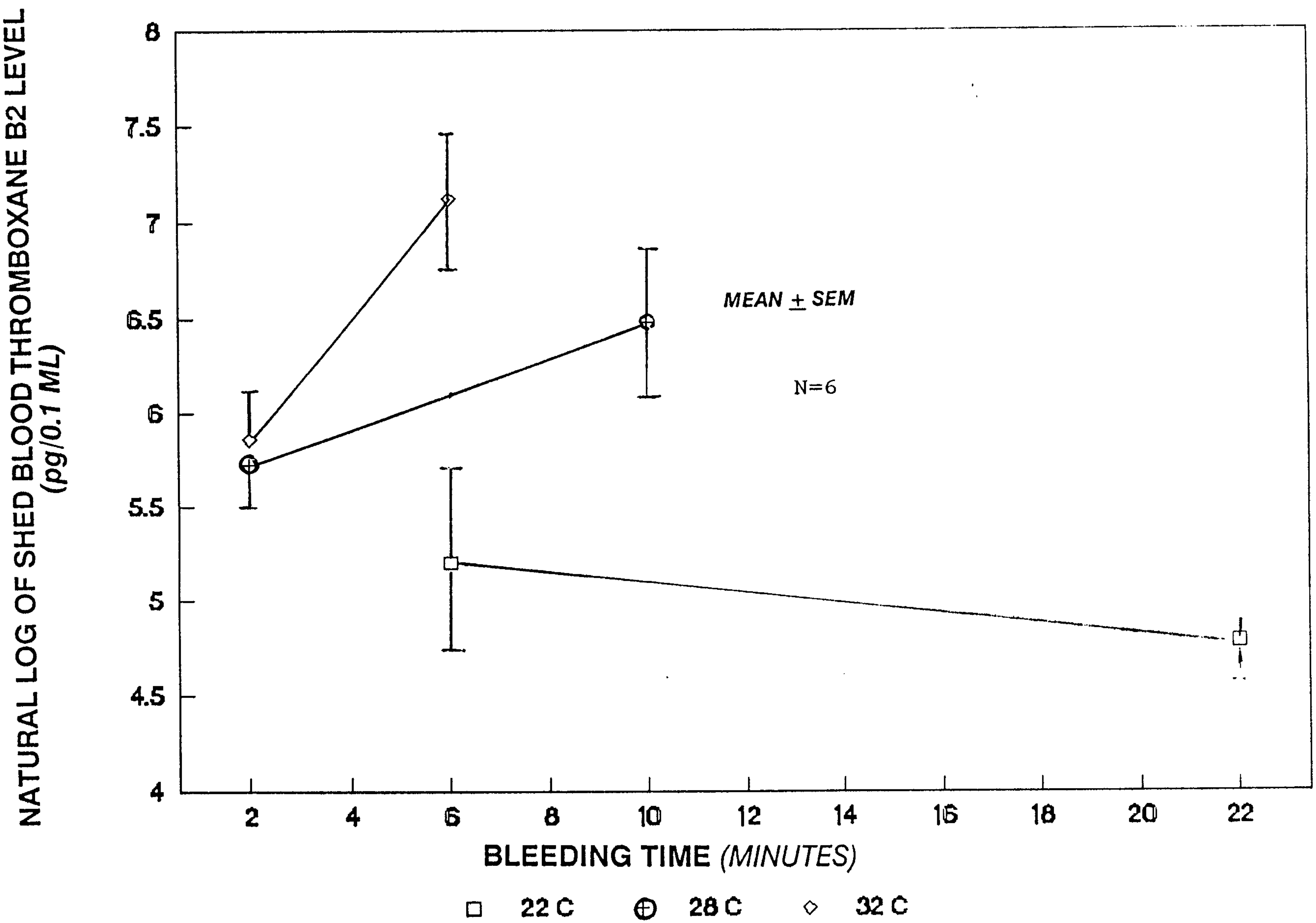

TABLE 6

IN VITRO CLOTTING TIMES AND THROMBOXANE B_2 LEVELS THE SERUM OBTAINED FROM 7 ML OF BLOOD CLOTTED WITH AGITATION IN 7.5 ML TUBES AT FOUR TEMPERATURES IN VITRO

n=3 Waterbath Temp	37C	32C	28C	22C
Clotting Time (min)				
1	3.5	4.5	7.5	15
2	3.0	3.5	6.5	15
3	5.0	5.5	6.5	13.5
Mean	3.8	4.5	6.8	14.5
SD	1.0	1.0	0.6	1.0
Serum TXB2 pg/.01 ml				
1	20200	1880	106	47
2	15000	1780	608	65
3	12200	3498	475	127
Mean	15800	2386	396	60
SD	4060	964	260	62

TABLE 7

IN VITRO CLOTTING TIMES AND THROMBOXANE B_2 LEVELS IN THE SERUM OBTAINED FROM 3 ML OF BLOOD CLOTTED WITH AGITATION IN 3.5 ML TUBES AT FOUR TEMPERATURES IN VITRO

n=3 Waterbath Temp	37C	32C	28C	22C	
Clotting Time (min)					
1			1	3.0	3.5
2	3.5	4.5	5.5	7.5	
3	3.5	4.5	5.5	8.0	
Mean	3.3	4.2	5.5	8.0	
SD	.3	.6	0	.5	
Serum TXB2 pg/.01 ml					
1	46789	13795	2565	509	
2	2385	640	1625	2301	
3	1863	1593	824	1170	
Mean	17000	5339	1338	1327	
SD	26000	7300	1067	906	

TABLE 8

N=2

IN VITRO CLOTTING TIMES AND THROMBOXANE B_2 LEVELS IN THE SERUM OBTAINED FROM 3.0 ML OF BLOOD CLOTTED WITH AND WITHOUT AGITATION IN A 3.5 ML TUBE AT FOUR TEMPERATURES IN VITRO

Waterbath Temp	37C	32C	28C	22C
Clotting Time (min)				
1	4.0	4.5	5.0	9.5
2	4.5	5.5	6.0	8.0
3	--	--	--	--
Mean	4.25	5.0	5.5	8.8
SD	.35	.7	.7	1.1

Serum TxB pg/.01 ml	A+	S++	A	S	A	S	A	S
1	2310	1015	2310	335	1503	629	657	297
2	1425	318	342	321	402	862	73	65
3	--	--	--	--	--	--	--	--
Mean	1868	667	1326	520	953	746	365	181
SD	620	493	1392	971	798	165	413	164

+Agitated for the measurement of clotting time
++Stationary for the period of time to clot the agitated sample

TABLE 9

IN VITRO CLOTTING TIMES AND THROMBOXANE B_2 LEVELS IN THE SERUM OBTAINED FROM 7 ML OF BLOOD CLOTTED WITH AND WITHOUT AGITATION IN A 7.5 ML TUBE AT FOUR TEMPERATURES IN VITRO

n=2

Waterbath Temp	37C	32C	28C	22C
Clotting Time (min)				
1	6.0	6.5	11.5	41.50+++
2	6.0	6.5	12.0	30.0
3	--	--	--	--
Mean	6.0	6.5	11.8	36
SD	0	0	.4	8

Serum TXB pg/.01 ml	A+	S++	A	S	A	S	A	S
1	3435	809	931	520	135	91	57	42
2	9615	931	6385	971	422	218	51	30
3	--	--	--	--	--	--	--	--
Mean	6525	890	3658	746	279	155	54	36
SD	4370	86	3857	319	203	90	4	8

+Agitated for the measurement of clotting time
++Stationary for the period of time to clot the agitated sample
+++ No clot formed

TABLE 10

IN VITRO CLOTTING TIMES AND THROMBOXANE B_2 LEVELS IN THE SERUM OBTAINED FROM 1 ML OF BLOOD CLOTTED WITH AND WITHOUT AGITATION IN A 3.5 ML TUBE AT FOUR TEMPERATURES IN VITRO

n=3

Waterbath Temp	37C	32C	28C	22C
Clotting Time (min)				
1	2.5	3.0	5.0	6.5
2	3.0	3.5	4.0	6.5
3	2.5	3.0	3.5	6.5
Mean	1.7	3.2	4.2	6.5
SD	1.0	0.7	0.8	0

Serum TXB pg/.01 ml	A+	S++	A	S	A	S	A	S
1	7373	3721	4000	1174	559	803	495	--
2	21320	4110	196000	2650	16170	1745	1853	1169
3	4694	598	1354	570	1234	512	2468	242
Mean	11129	2810	8318	1464	5988	1020	1605	
SD	8926	1925	9860	1070	8825	645	1010	

+Agitated for the measurement of clotting time
++Stationary for the period of time to clot the agitated sample

TABLE 11

IN VITRO CLOTTING TIMES AND THROMBOXANE B2 LEVELS IN THE SERUM OBTAINED FROM 1 ML OF BLOOD CLOTTED WITH AND WITHOUT AGITATION IN A 3.5 ML TUBE AT FOUR TEMPERATURES IN VITRO

n=3

Waterbath Temp	37C	32C	28C	22C
Clotting Time (min)				
1	2.5	3.0	3.5	6.0
2	3.0	4.0	5.0	6.5
3	2.5	3.5	4.5	5.0
Mean	2.7	3.5	4.3	5.8
SD	.3	.5	.8	.8

Waterbath Temp	37C		32C		28C		22C	
Serum TXB pg/.01 ml	A+	S++	A	S	A	S	A	S
1	2958	559	1232	259	806	246	447	256
2	5958	828	3810	311	1171	250	354	125
3	5520	603	1336	228	820	610	611	250
Mean	4812	663	2126	266	932	369	471	177
SD	1620	144	1459	42	207	209	130	132

\+ Agitated for the measurement of clotting time
++Stationary for the period of time to clot the agitated sample

TABLE 12

IN VITRO CLOTTING TIME AND THROMBOXANE B_2 LEVELS IN THE SERUM OBTAINED FROM 1.0 ML OF BLOOD CLOTTED WITH AND WITHOUT AGITATION IN A 3.5 ML TUBE AT FOUR TEMPERATURES IN VITRO

n=6	Waterbath Temp	37C	32C	28C	22C
	Clotting Time (min)	2.5	3.0	5.0	6.5
		3.0	3.5	4.0	6.5
		2.5	3.0	3.5	6.5
		2.5	3.0	3.5	6.0
		3.0	4.0	5.0	6.5
		2.5	3.5	4.5	5.0
	Mean:	2.7	3.33	4.3	6.2
	SD:	.3	.4	.7	.6

Serum TXB pg/.01 ml	A+	S++	A	S	A	S	A	S
	7373	3721	4000	1174	559	803	495	--
	21302	4110	19600	2650	16170	1745	1853	1169
	4694	598	1354	570	1234	512	2468	242
	2958	559	1232	259	806	246	447	256
	5958	828	3810	311	1171	250	354	125
	5520	603	1336	228	820	610	611	250
Mean:	7967	1737	5222	865	3460	694	1038	389
SD:	6693	1695	7158	943	6232	558	895	447

\+ Agitated for the measurement of clotting time.
++ Stationary for the period of time to clot the agitated sample.

TABLE 13

IN VITRO CLOTTING TIMES AND THROMBOXANE B_2 LEVELS IN THE SERUM OBTAINED FROM 1 ML OF BLOOD CLOTTED WITH AGITATION IN A 3.5 ML TUBE AT FOUR TEMPERATURES IN VITRO

Waterbath Temp	37C	32C	28C	22C
Clotting Time (min)				
Mean:	2.7	3.33	4.3	6.2
SD:	0.3	0.4	0.7	0.6
n:	6	6	6	6
Serum TXB pg/.01 ml				
Mean:	7967	5222	3460	1038
SD:	6693	7158	6232	895
n:	6	6	6	6

TABLE 14A

THE IN VITRO CLOTTING TIME AND THROMBOXANE B2 LEVELS IN THE SERUM OBTAINED FROM AGITATED OR NON-AGITATED WHOLE BLOOD CLOTTED AT FOUR TEMPERATURES

Waterbath Temp	37C	32C	28C	22C	1 Way AOV
Clotting Time (min)					
Mean	3.6	4.3	6.1	12.1	<.0001
SD	1.2	1.2	2.5	10.0	
n	16	16	16	16	
Serum TXB2 pg/0.1 ml from agitated whole blood					
Mean	10190	4030	1839	705	<.0001
SD	11650	5286	3873	812	
n	16	16	16	16	
Serum TXB2 pg/0.1 ml from non-agitated whole blood					
Mean	1349	734	597	275	NS
SD	1371	744	481	350	
n	10	10	10	10	
Paired T between agitated whole blood and non-agitated whole blood	<.01	<.05	NS	NS	

FIGURE 33

THE RELATIONSHIP BETWEEN THE CLOTTING TIME IN THE AGITATED WHOLE BLOOD AND FOUR BLOOD TEMPERATURES

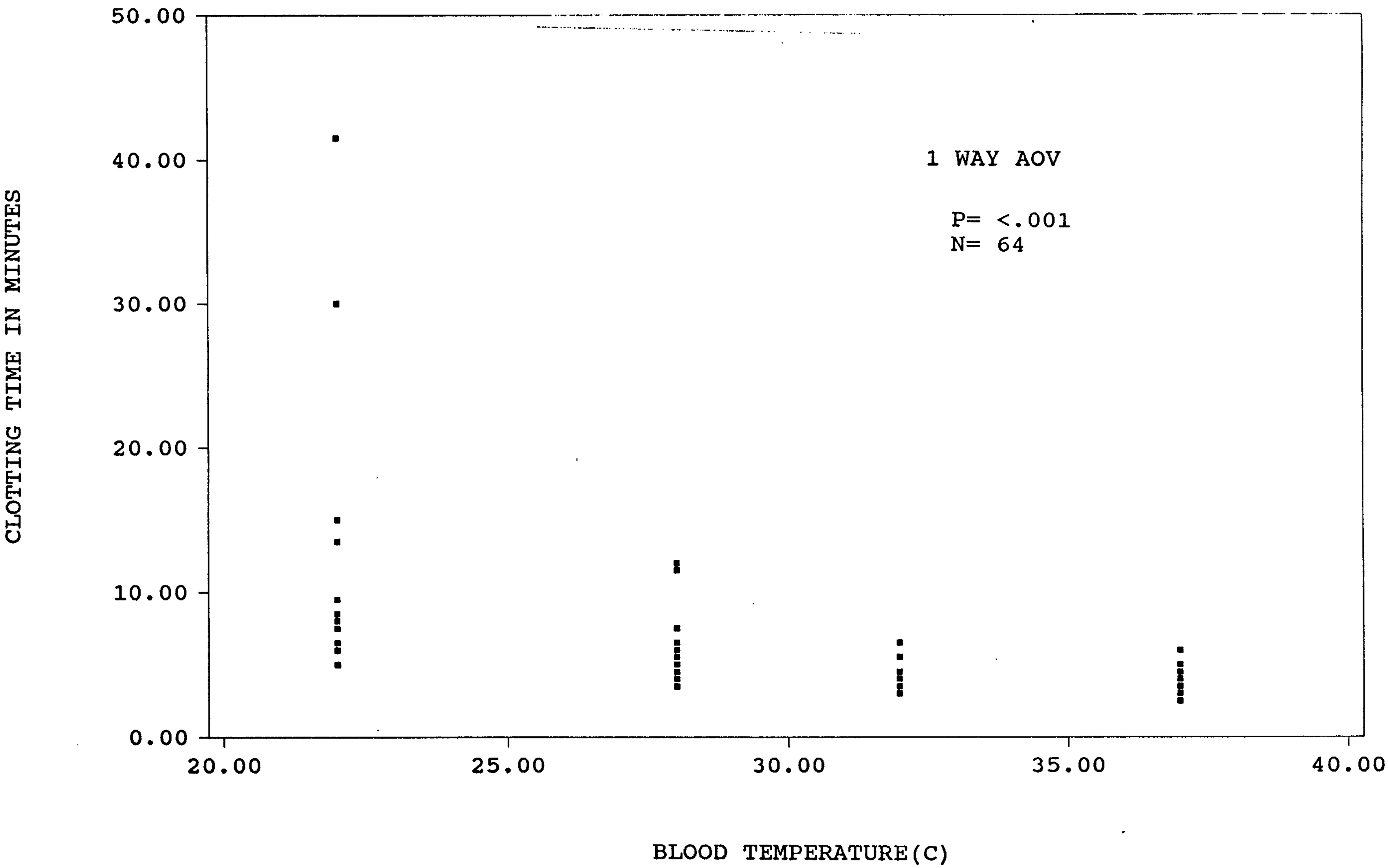

FIGURE 34

THE RELATIONSHIP OF THE SERUM THROMBOXANE B_2 LEVELS IN AGITATED CLOTTED BLOOD AND FOUR BLOOD TEMPERATURES

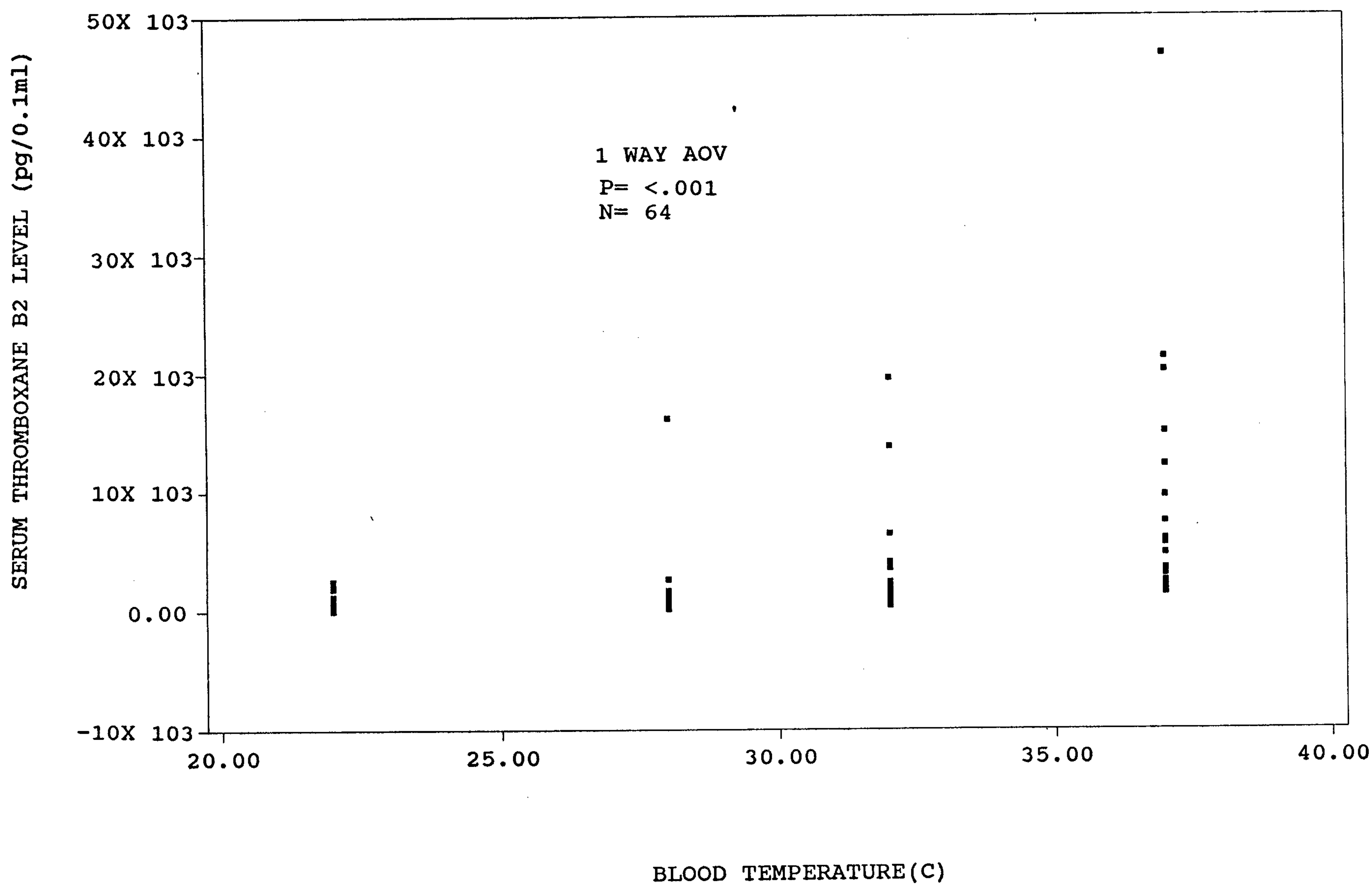

FIGURE 35

THE RELATIONSHIP BETWEEN THE SERUM THROMBOXANE B_2 LEVELS IN NON AGITATED CLOTTED BLOOD AND FOUR BLOOD TEMPERATURES

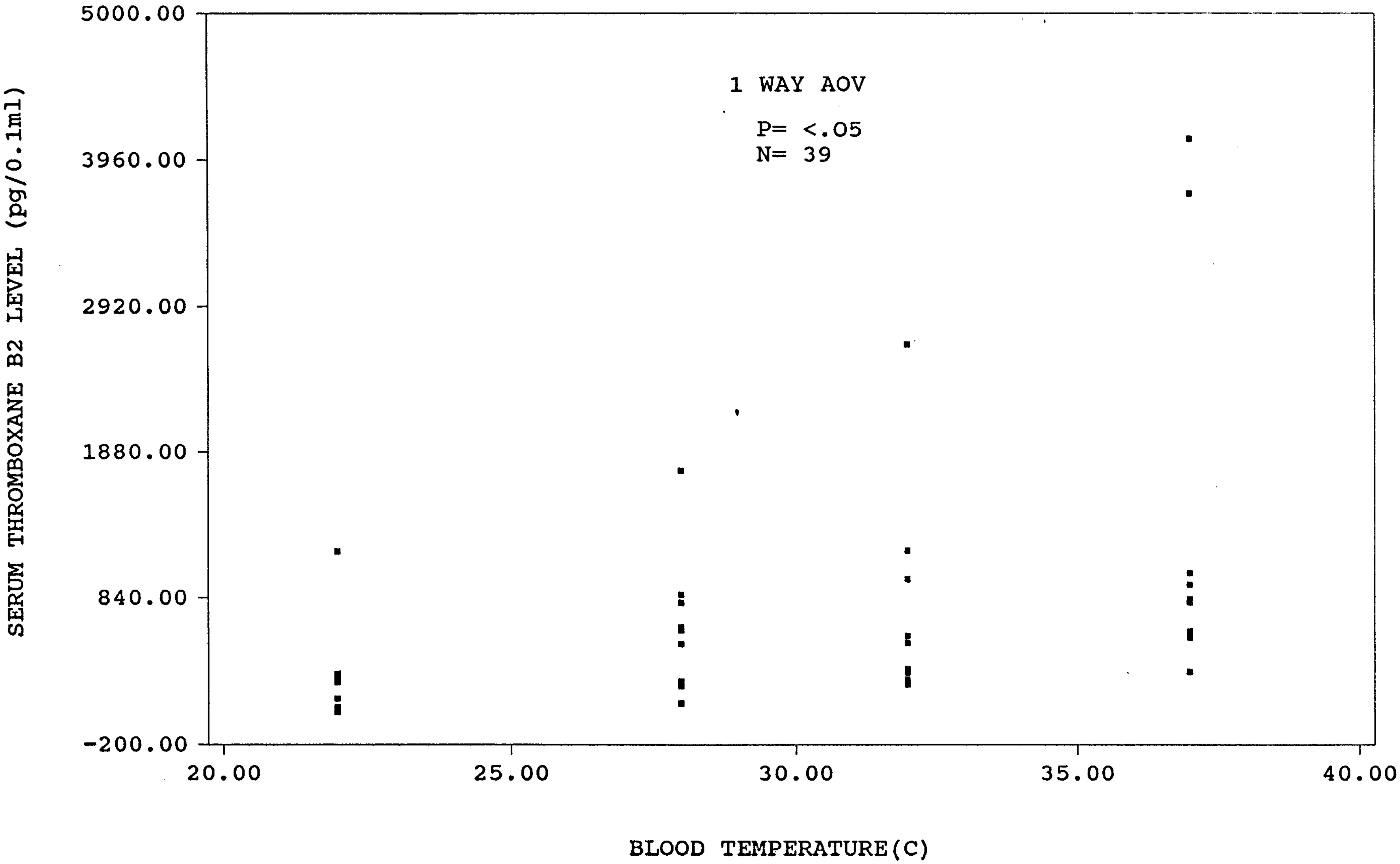

FIGURE 36

THE CLOTTING TIME IN MINUTES OF AGITATED WHOLE BLOOD AT FOUR BLOOD TEMPERATURES

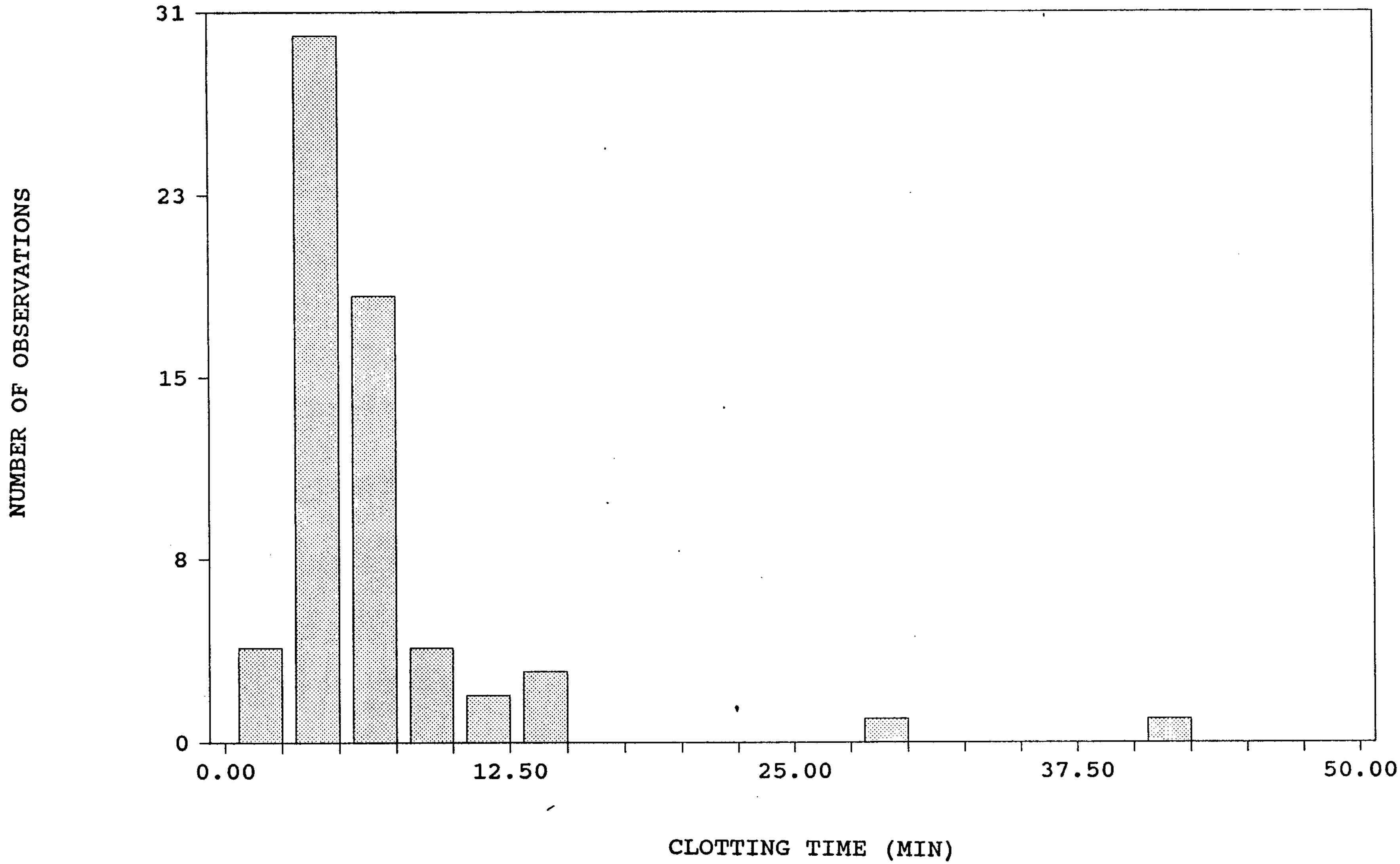

FIGURE 37

THE THROMBOXANE B_2 LEVELS IN THE SERUM OF AGITATED CLOTTED WHOLE BLOOD AT FOUR BLOOD TEMPERATURES

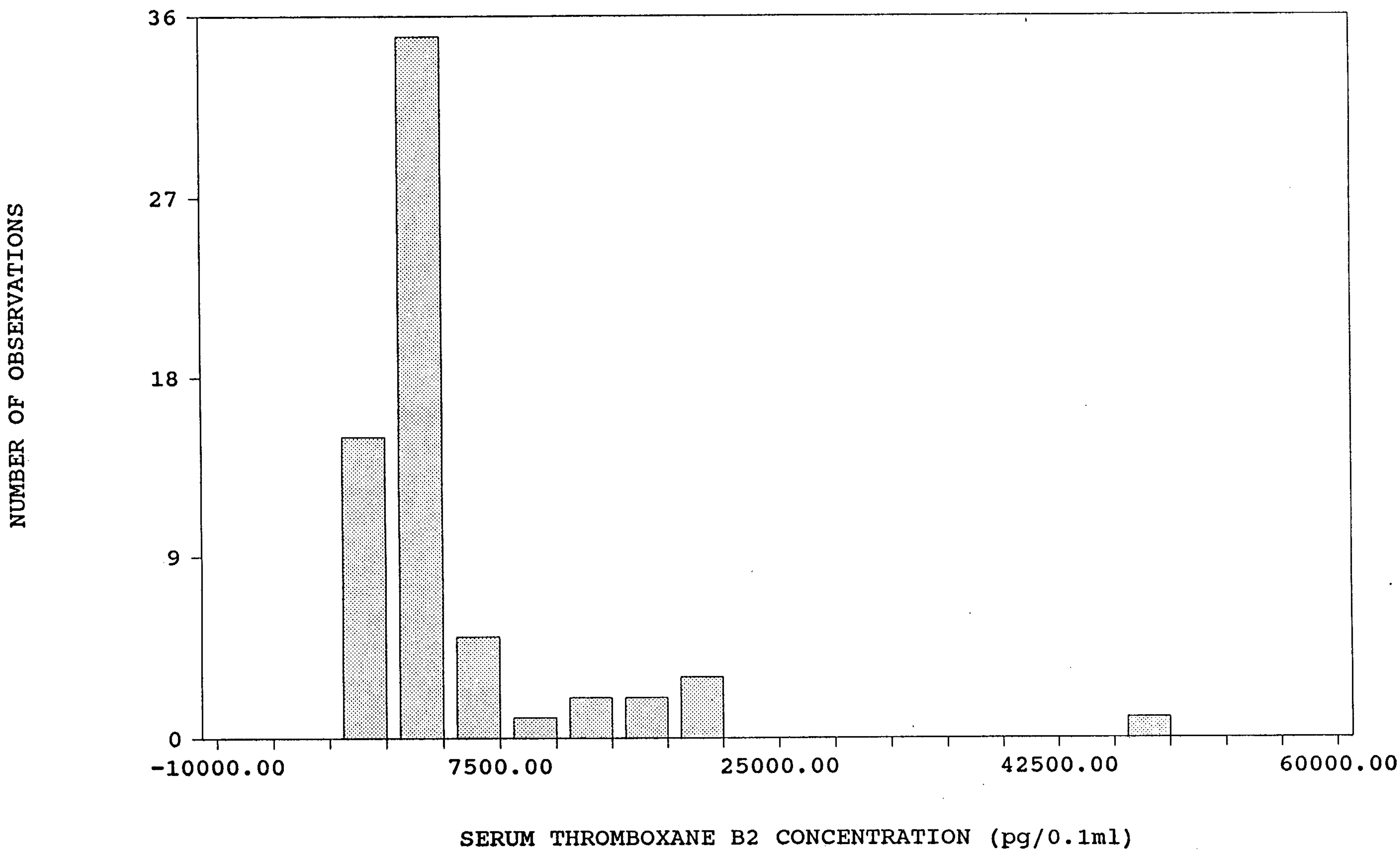

FIGURE 38

THE THROMBOXANE B_2 LEVELS IN THE SERUM OF NON AGITATED CLOTTED WHOLE BLOOD AT FOUR BLOOD TEMPERATURES

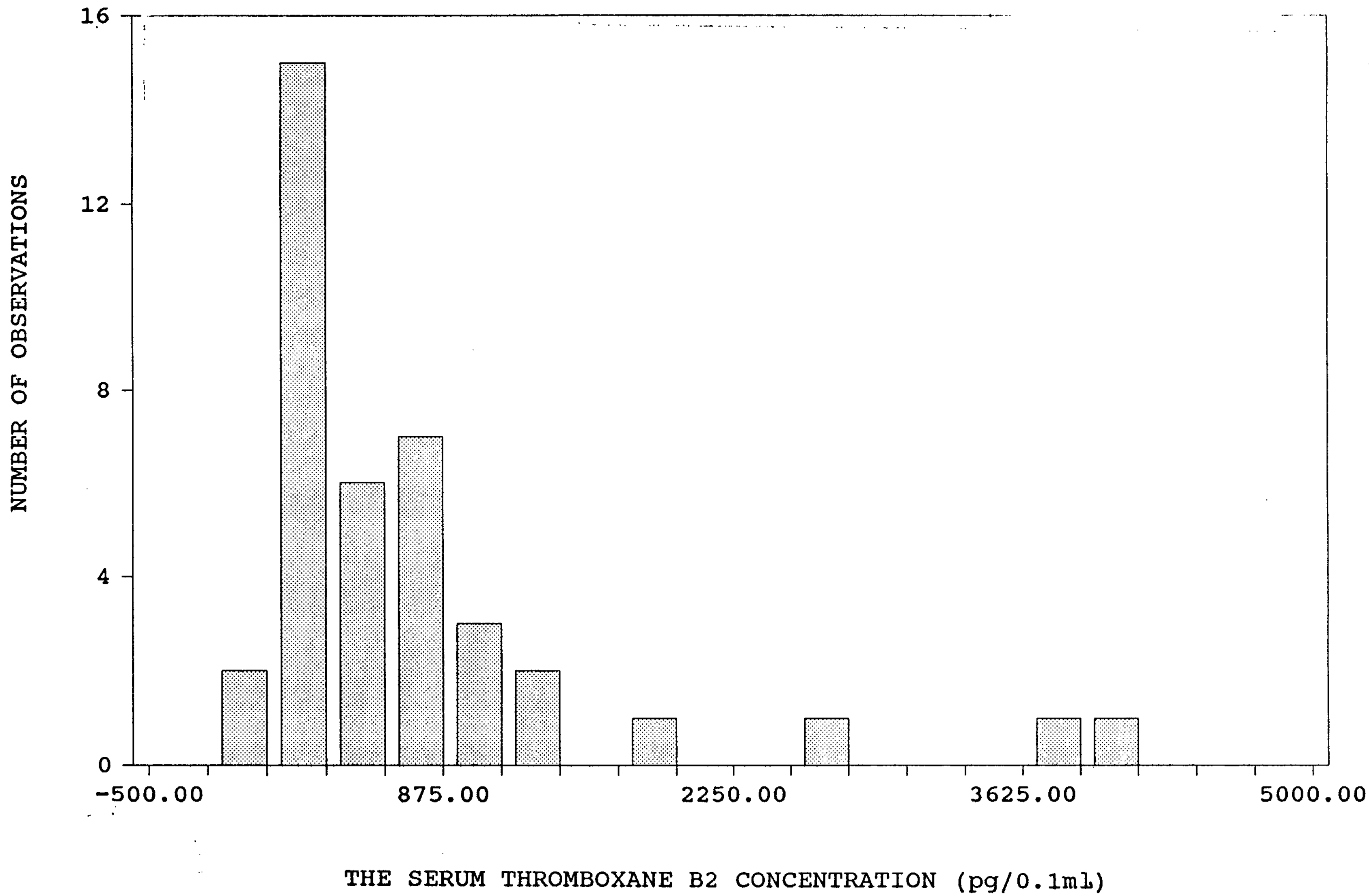

TABLE 14B

THE NATURAL LOGARITHM OF THE IN VITRO CLOTTING TIME AND THROMBOXANE B_2 LEVEL IN THE SERUM OBTAINED FROM AGITATED OR NON-AGITATED WHOLE BLOOD CLOTTED AT FOUR TEMPERATURES

Waterbath Temp	37C	32C	28C	22C	1 Way AOV
Clotting Time (min)					
Mean	1.24	1.4	1.74	2.28	<.0001
SD	.3	.3	.3	.6	
n	16	16	16	16	
Serum TXB2 pg/0.1 ml from agitated whole blood					
Mean	8.7	7.7	6.7	5.8	<.0001
SD	1.0	1.0	1.1	1.4	
n	16	16	16	16	
Serum TXB2 pg/0.1 ml from non-agitated whole blood					
Mean	6.9	6.3	6.1	5.1	<.01
SD	.8	.8	.8	1.0	
n	10	10	10	10	
Paired T between agitated whole blood and non-agitated whole blood	<.001	<.001	NS	NS	

FIGURE 39

THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE CLOTTING TIME IN AGITATED WHOLE BLOOD AND FOUR BLOOD TEMPERATURES

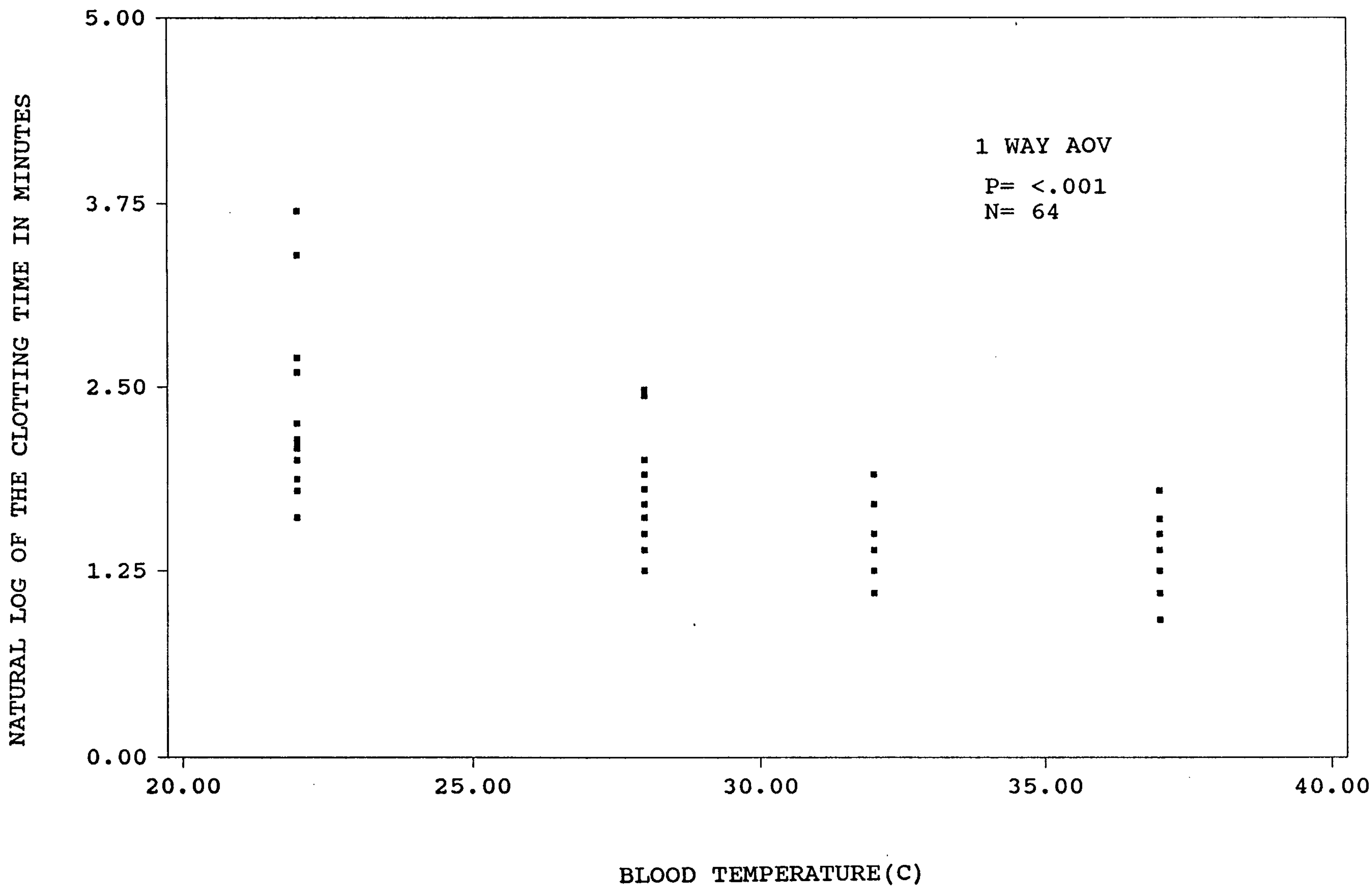

FIGURE 40

THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE SERUM THROMBOXANE B_2 LEVEL IN AGITATED CLOTTED BLOOD AT FOUR BLOOD TEMPERATURES

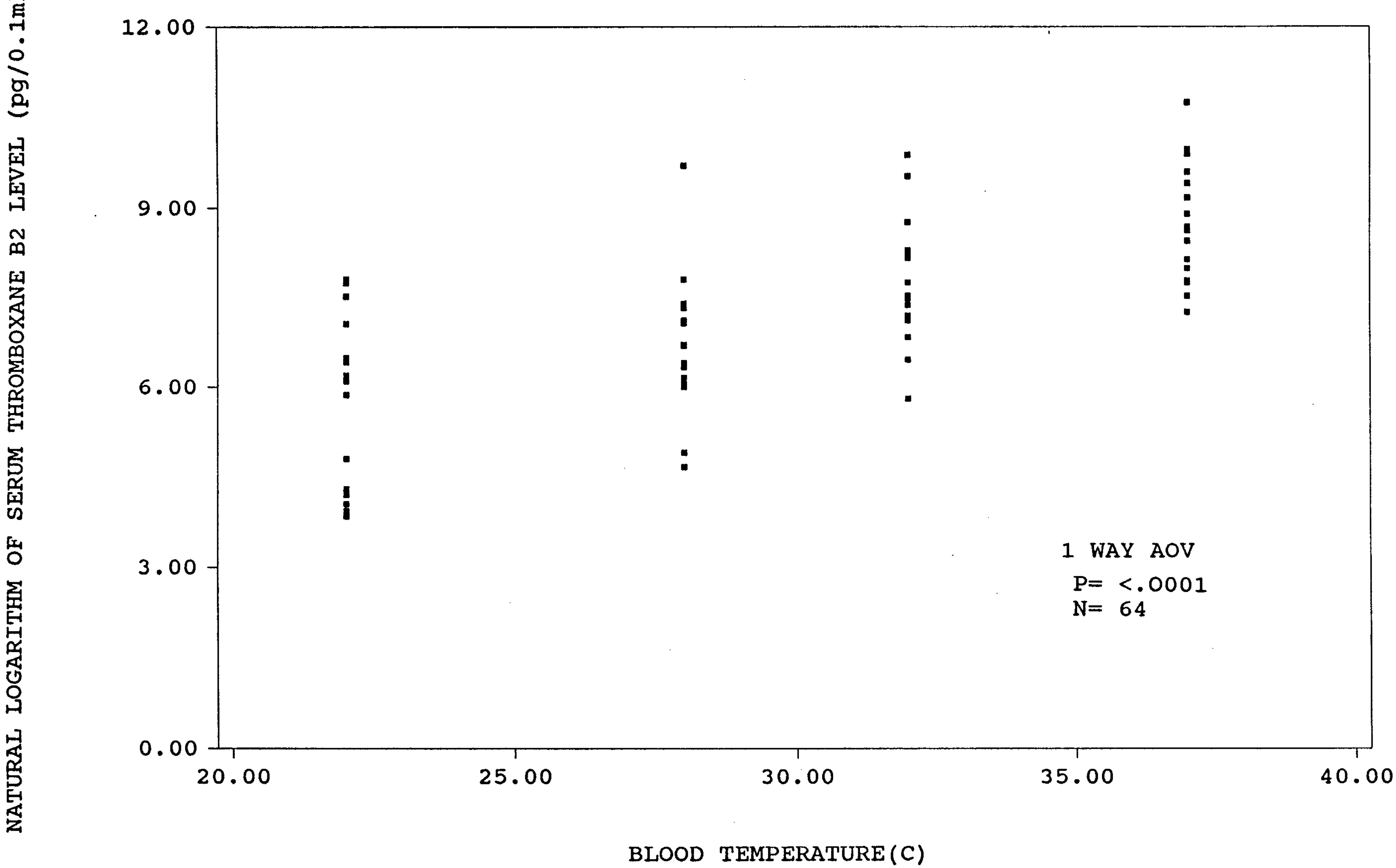

FIGURE 41

THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE SERUM THROMBOXANE B_2 LEVELS IN NON AGITATED CLOTTED BLOOD AND FOUR BLOOD TEMPERATURES

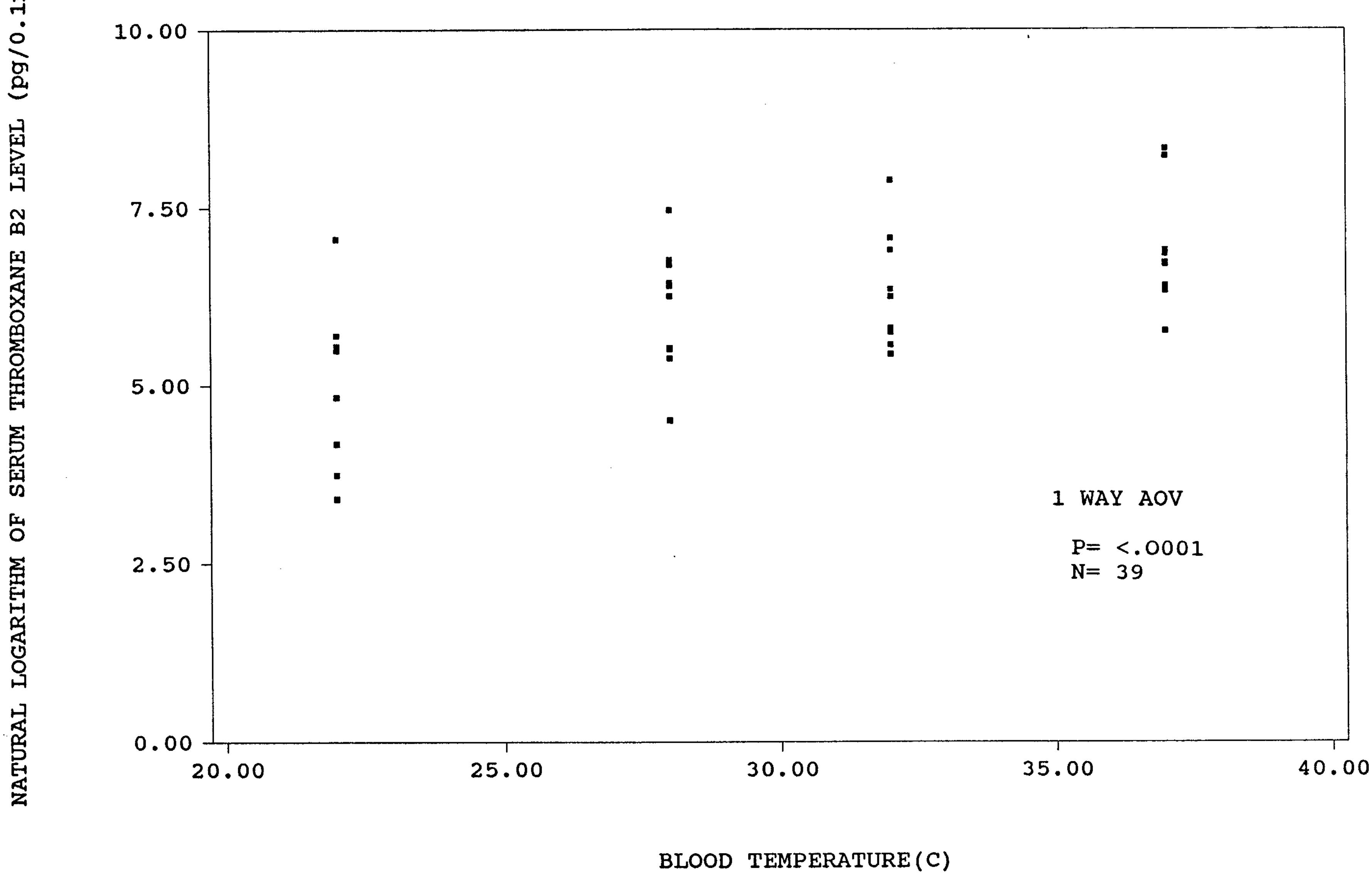

FIGURE 42

THE NATURAL LOGARITHM OF THE CLOTTING TIME OF AGITATED WHOLE BLOOD AT FOUR BLOOD TEMPERATURES

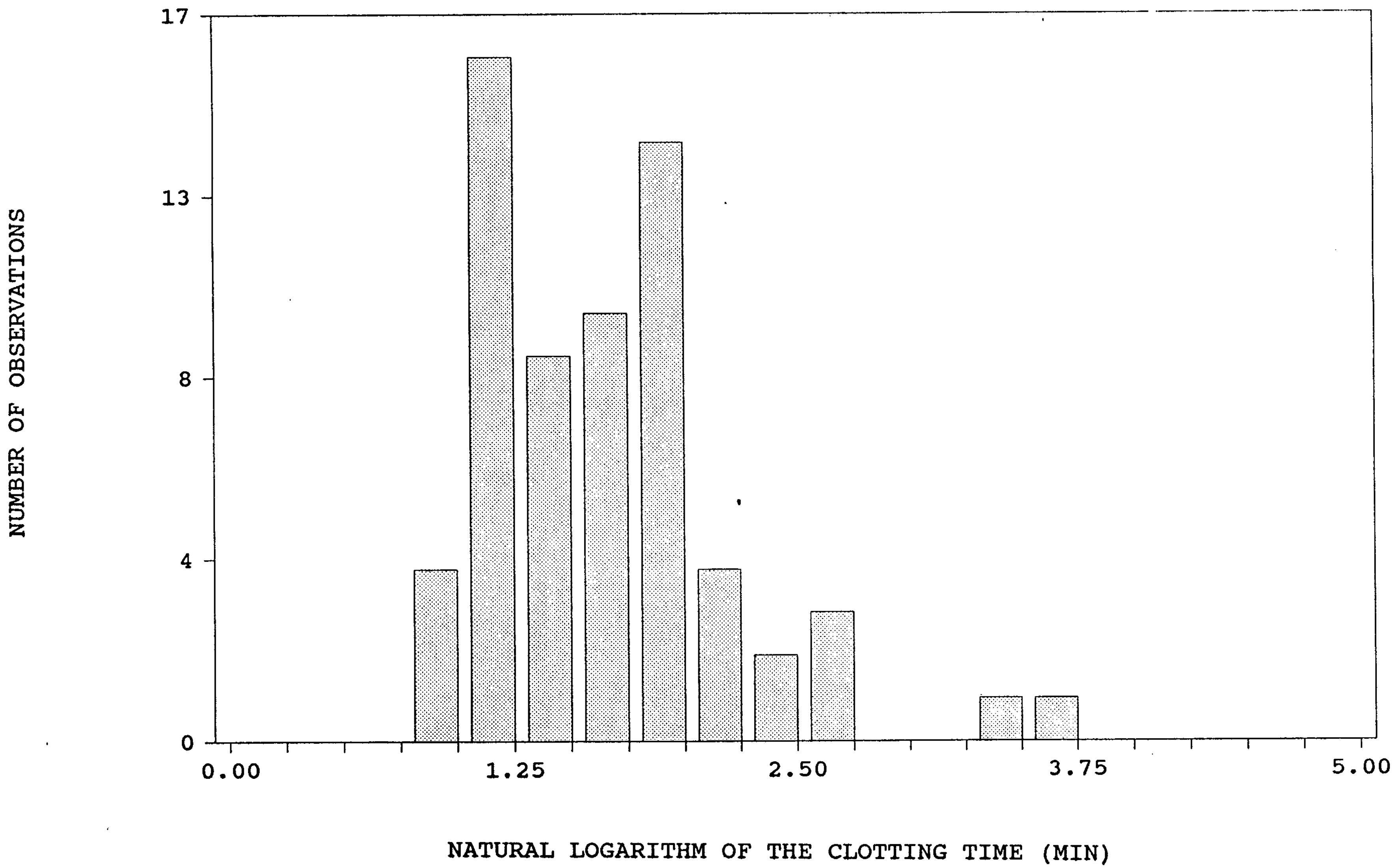

FIGURE 43

THE NATURAL LOGARITHM OF THE THROMBOXANE B_2 LEVEL IN SERUM OF AGITATED CLOTTED WHOLE BLOOD AT FOUR BLOOD TEMPERATURES

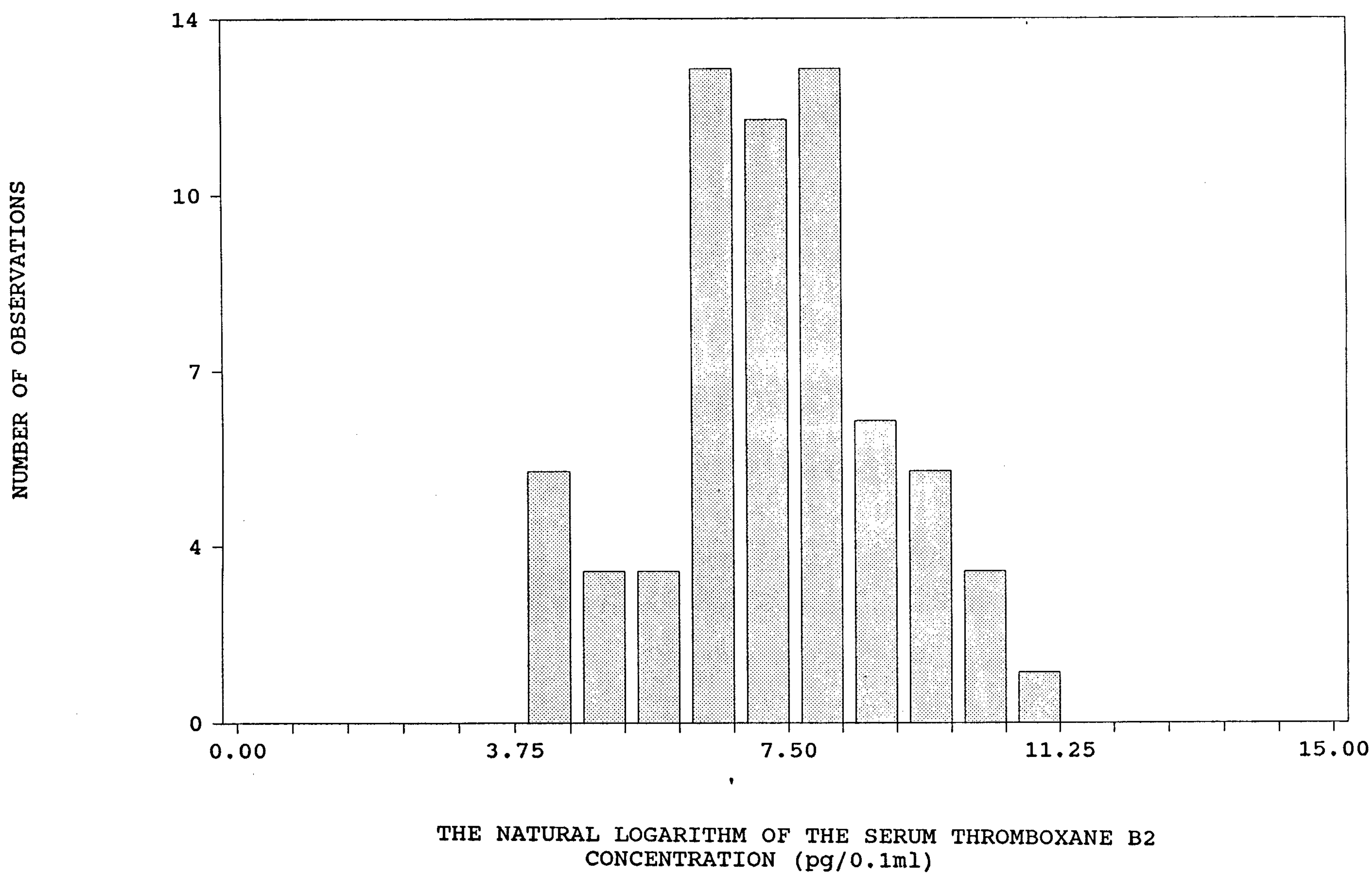

FIGURE 44

THE NATURAL LOGARITHM OF THE THROMBOXANE B_2 LEVEL IN THE SERUM OF NON AGITATED CLOTTED WHOLE BLOOD AT FOUR BLOOD TEMPERATURES

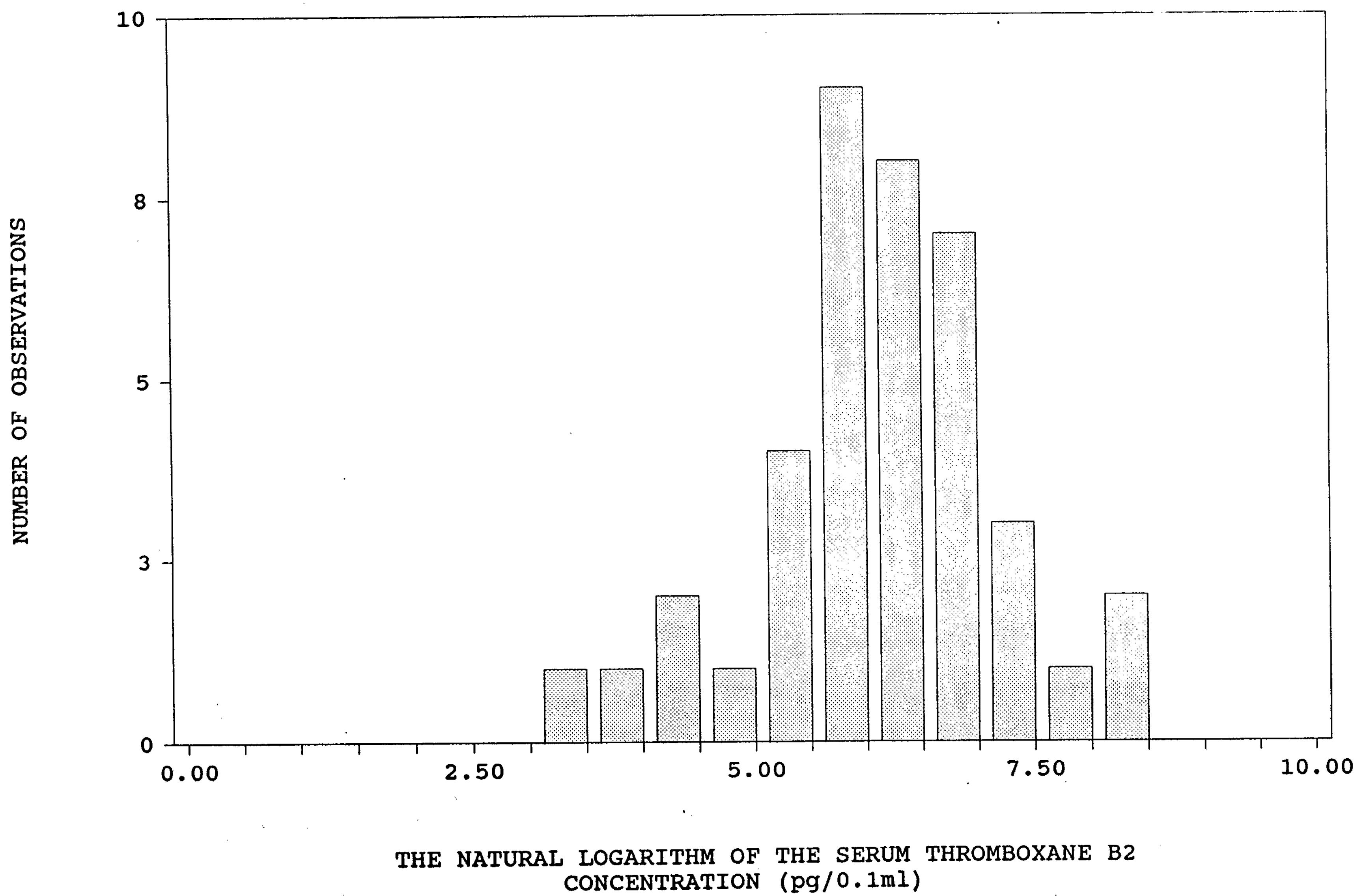

TABLE 15A

THROMBOXANE B_2 LEVELS AT 30 SECOND INTERVALS IN THE SERUM SEPARATED FROM CLOTTED BLOOD MAINTAINED AT FOUR TEMPERATURES; ONE ML OF BLOOD AS COLLECTED INTO A 3.5 ML PLASTIC TUBE AND AGITATED EVERY 30 SECONDS

N=3 TIME IN MINUTES

Temp		30'	1 Min	1'30"	2'	2'30"	1 WAY ANOVA
		TXB2 Level (pg/0.1 ml)					
37C							
	1.	64	365	648	869	2696	
	2.	329	207	767	2761	6765	
	3.	56	100	143	351	747	
	Mean:	150	224	519	1327	3403	NS
	SD:	155	133	331	1268	3070	
32 C							
	1.	277	751	1464	927	2613	
	2.	227	824	1383	1380	1585	
	3.	138	112	630	998	1160	
	Mean:	214	562	1159	1102	1786	<.05
	SD:	70	392	460	244	747	
28 C							
	1.	37	148	95	188	307	
	2.	50	102	37	57	257	
	3.	22	54	48	90	72	
	Mean:	36	101	60	112	212	NS
	SD:	14	47	31	68	124	
22 C							
	1.	46	75	128	104	235	
	2.	34	48	37	57	33	
	3.	29	29	61	36	49	
	Mean:	36	51	75	66	106	NS
	SD:	9	23	47	35	112	
1 Way ANOVA:		NS	NS	<.01	NS	NS	

TABLE 15B

NATURAL LOGARITHM OF THE THROMBOXANE B_2 LEVEL AT 30 SECOND INTERVALS IN THE SERUM SEPARATED FROM CLOTTED BLOOD MAINTAINED AT FOUR TEMPERATURES; ONE ML OF BLOOD WAS COLLECTED INTO A 3.5 ML PLASTIC TUBE AND AGITATED EVERY 30 SECONDS

n=3

TIME IN MINUTES

Temp		30'	1 Min	1'30"	2'	2'30"	1 Way ANOVA
		TXB2 Level (pg/0.1 ml)					
37C							
	1.	4.16	5.9	6.47	6.77	7.9	
	2.	5.8	5.53	6.64	7.92	8.82	
	3.	4.03	4.61	4.96	5.86	6.62	
	Mean:	4.66	5.28	6.03	6.85	7.78	<.05
	SD:	1.0	.7	.9	1.0	1.1	
32 C							
	1.	5.62	6.62	7.29	6.83	7.87	
	2.	5.42	6.71	7.23	7.23	7.37	
	3.	4.93	4.72	6.45	6.91	7.06	
	Mean:	5.33	6.02	6.99	6.99	7.43	<.01
	SD:	.4	1.1	.5	.2	.4	
28 C							
	1.	3.61	5.0	4.55	5.24	5.73	
	2.	3.91	4.62	3.61	4.04	5.55	
	3.	3.09	3.99	3.87	4.5	4.28	
	Mean:	3.54	4.54	4.01	4.59	5.18	<.05
	SD:	.4	.5	.5	.6	.8	
22 C							
	1.	3.83	4.32	4.85	4.64	5.46	
	2.	3.53	3.87	3.61	4.04	3.5	
	3.	3.37	3.37	4.11	3.58	3.89	
	Mean:	3.57	3.85	4.19	4.09	4.28	NS
	SD:	.23	.5	.6	.5	1.0	
1 Way ANOVA:		<.05	<.05	<.01	<.01	<.01	

TABLE 16

THE RATE OF THE THROMBOXANE B_2 PRODUCTION AND THE NATURAL LOGARITHM OF THE RATE OF THE THROMBOXANE B_2 PRODUCTION IN BLOOD CLOTTED WITH AGITATION AT 37C, 32C, 28C, AND 22C AND IN SHED BLOOD FROM THE BLEEDING TIME SITE WHERE THE FOREARM TEMPERATURE WAS 32C, 28C, AND 22C

EMPERATURE degrees C)	RATE OF THROMBOXANE PRODUCTION (PG/ML/SECOND)		NATURAL LOG OF THE RATE OF THROMBOXANE PRODUCTION (PG/ML/SECOND)	
	clotted blood N=16	shed blood N=6	clotted blood N=16	shed blood N=6
37				
MEAN	525		5.7	
SE	162		1.1	
32				
MEAN	175	21.2	4.5	2.8
SE	63	6.5	1.1	.7
28				
MEAN	69	7.49	3.2	1.8
SE	40	2.0	1.5	.7
22*				
MEAN	17	.9	1.7	.4
SE	5	.3	1.0	.9
WAY AOV .0001	<.01	<.05	<.001	<.001

N=5

FIGURE 45

RATE of TXB2 PRODUCTION
Clotted Blood

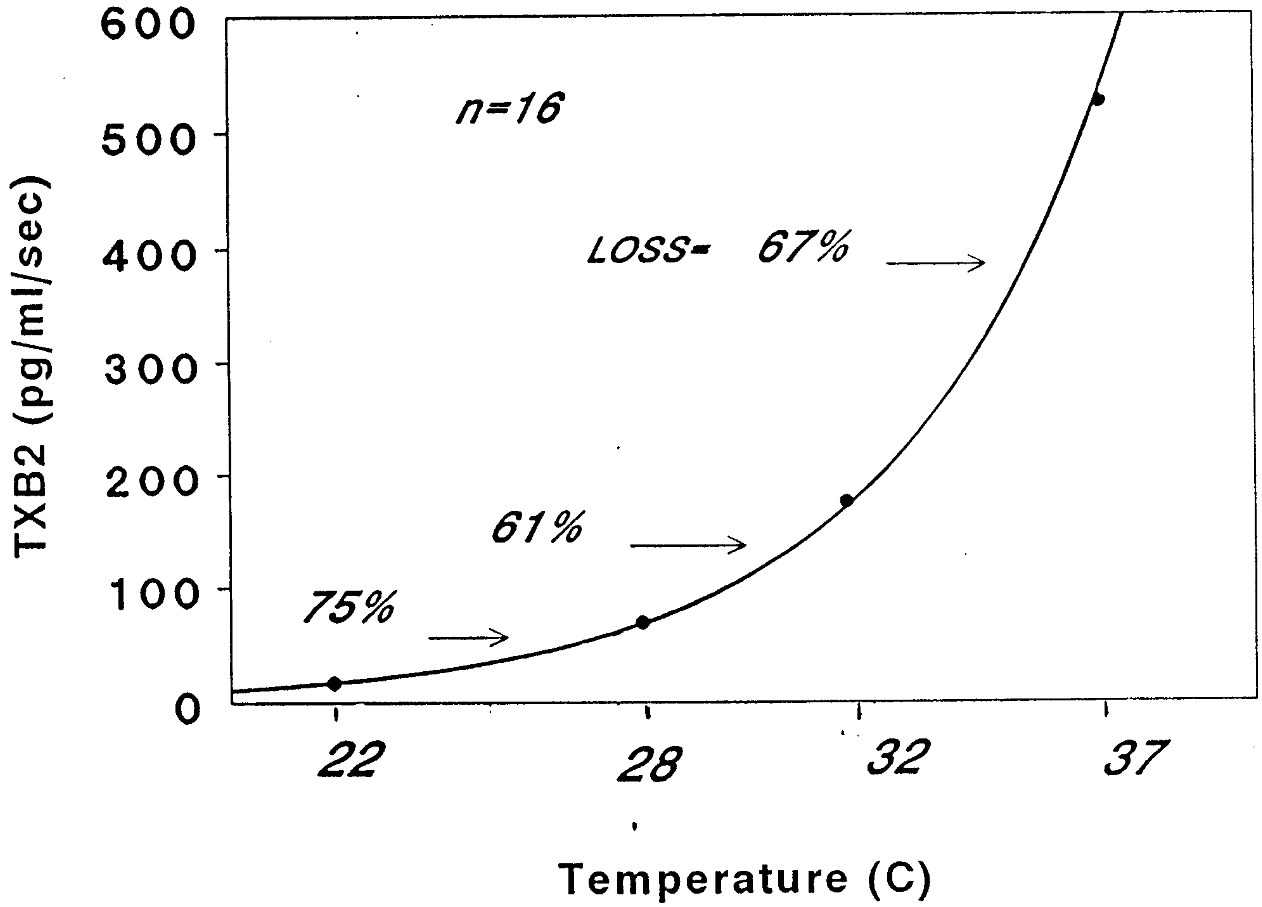

FIGURE 46

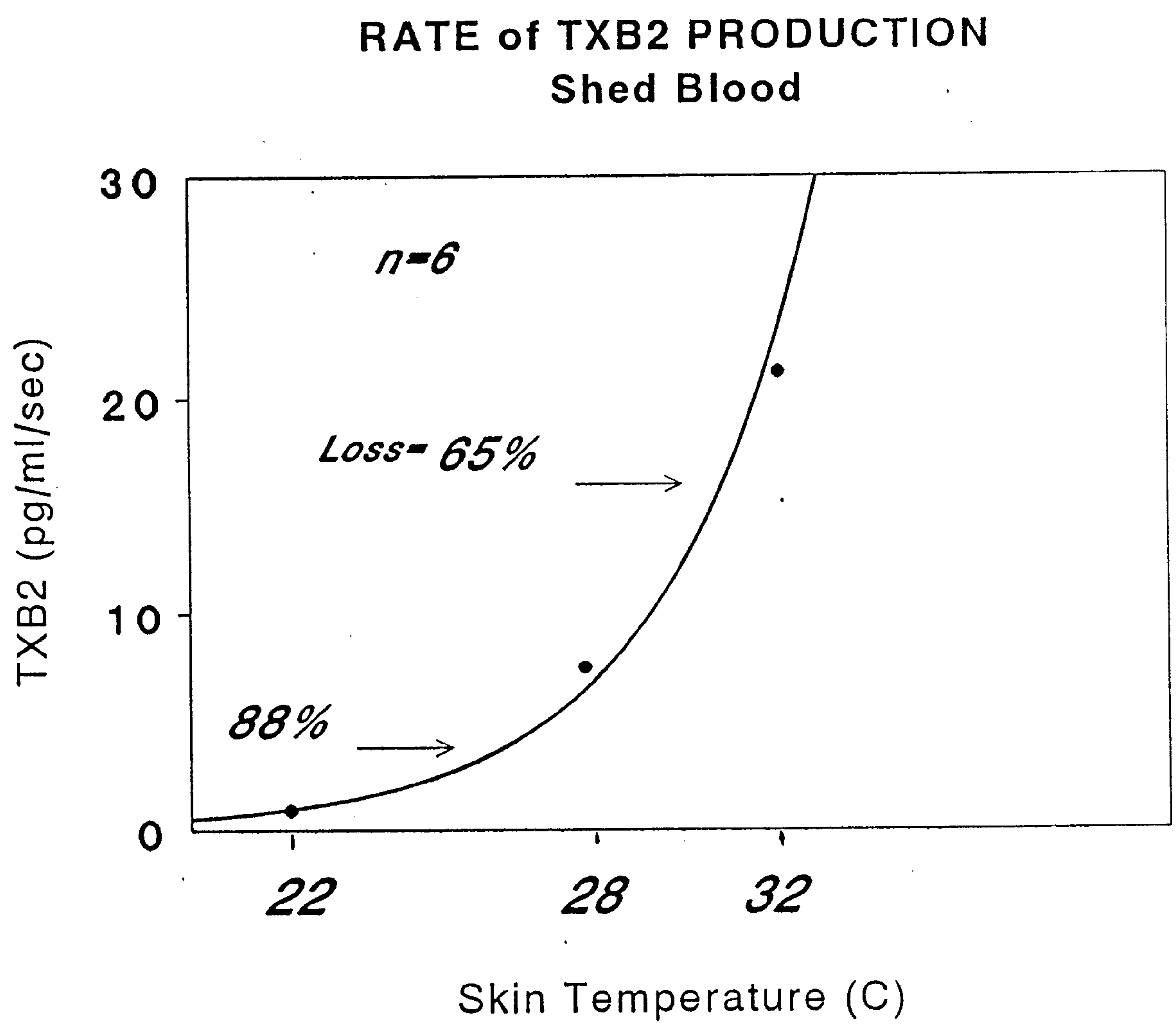
RATE of TXB2 PRODUCTION
Shed Blood
30
20
10
0
TXB2 (pg/ml/sec)
n=6
Loss= 65%
88%
22
28
32
Skin Temperature (C)

FIGURE 47

THE RATE OF THROMBOXANE B_2 PRODUCTION IN AGITATED CLOTTED BLOOD MAINTAINED AT +22C, +28C, +32C, AND +37C

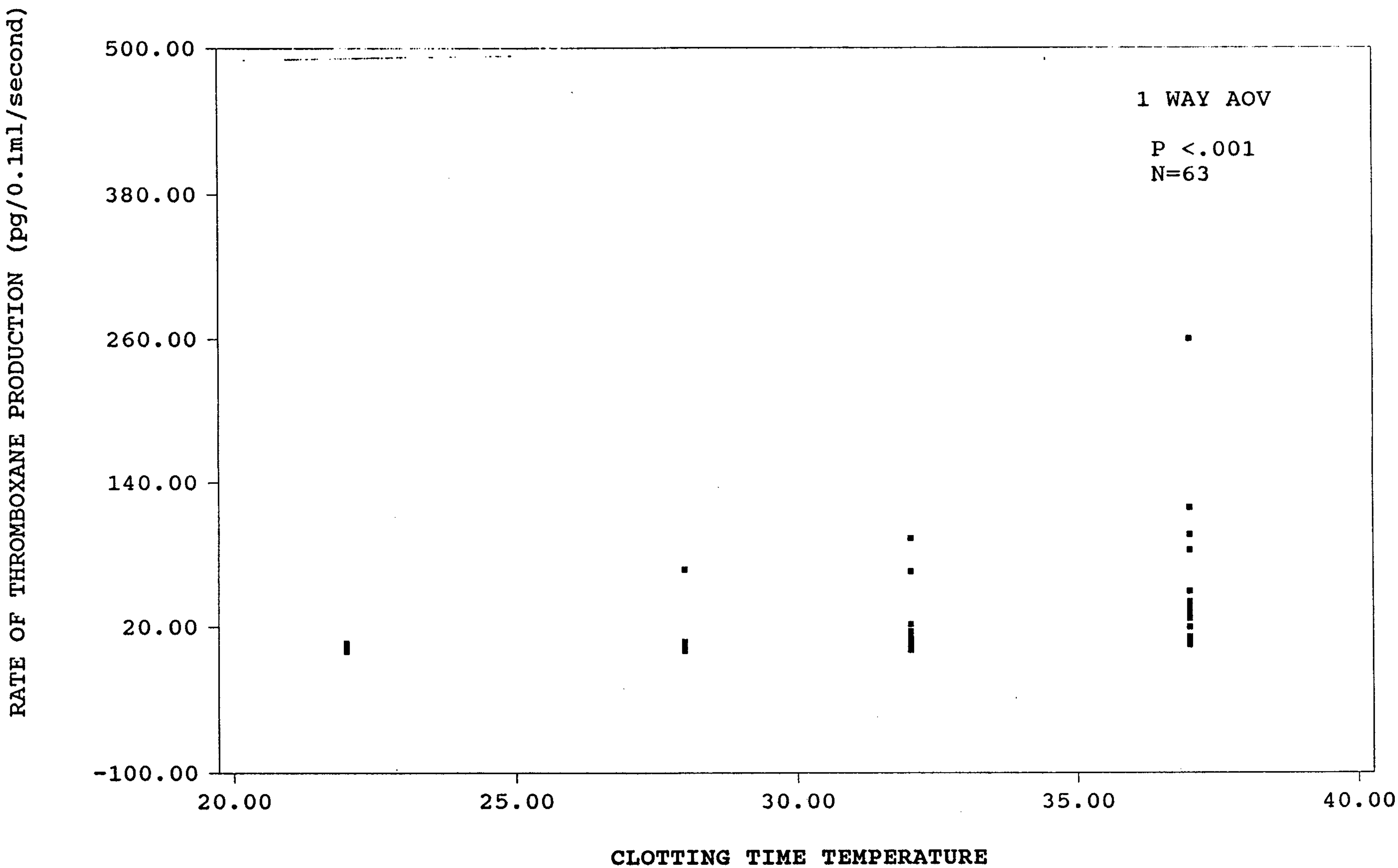

FIGURE 48

THE NATURAL LOGARITHM OF THE RATE OF THE THROMBOXANE B_2 PRODUCTION IN BLOOD CLOTTED WITH AGITATION AT 22C, 28C, 32C, AND 37C

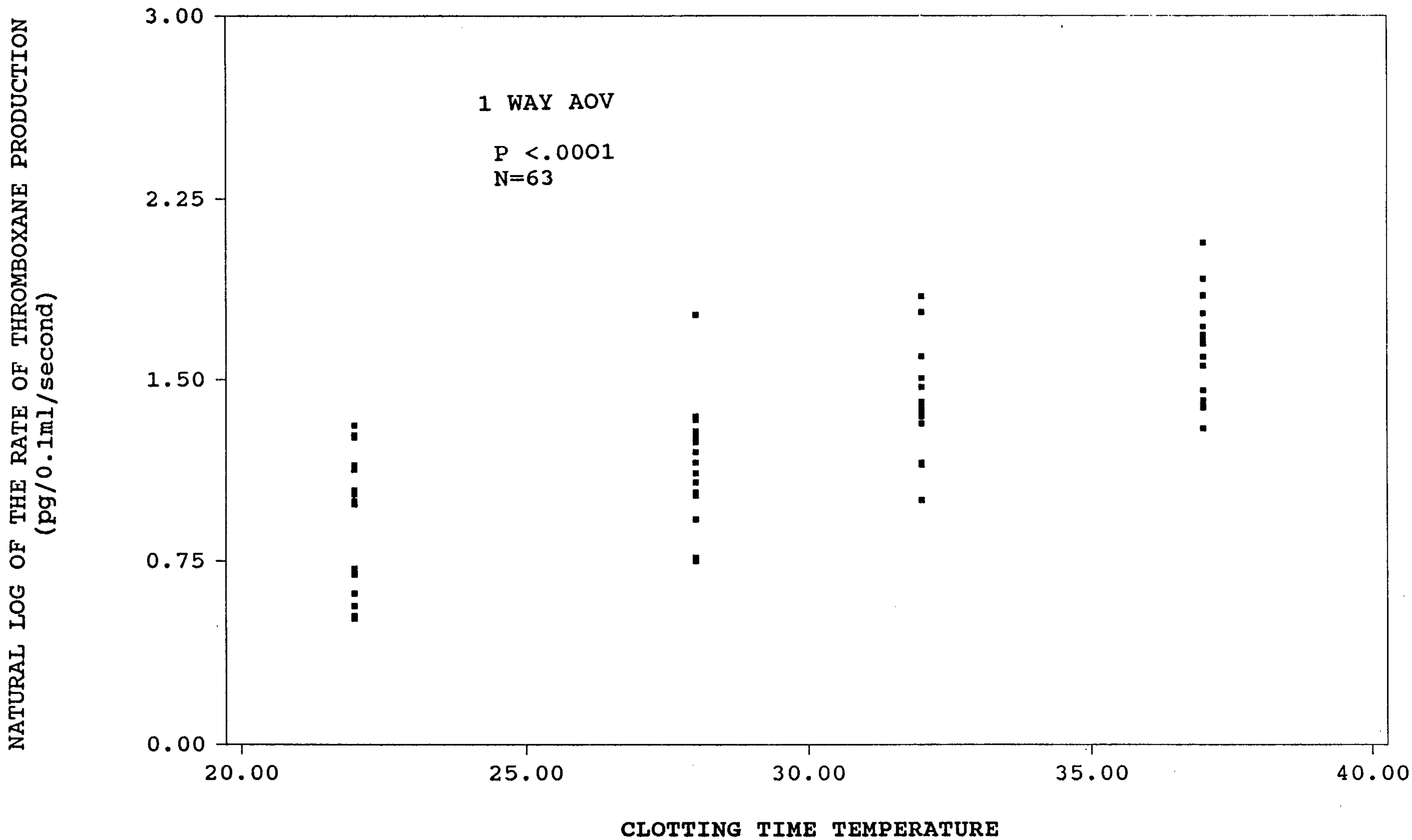

TABLE 17A

BLEEDING TIME AND TOTAL HEMOGLOBIN IN THE SHED BLOOD COLLECTED ON THE FILTER PAPER IN 16 NORMAL VOLUNTEERS AT SEVEN SKIN TEMPERATURES

Temperature (C)		Bleeding Time (Min)	Total Hemoglobin Collected	Correlation Coefficient R	P
38					
	Mean:	5.3	82		
	SD:	1.5	62		
	n:	16	16	.764	<.01
35					
	Mean:	5.3	61		
	SD:	1.6	43		
	n:	16	16	.763	<.05
32					
	Mean:	6.5	50		
	SD:	1.9	65		
	n:	16	16	.740	<.01
29					
	Mean:	10.5	71		
	SD:	2.8	36		
	n:	16	16	.070	NS
26					
	Mean:	12.0	73		
	SD:	3.6	33		
	n:	16	16	.574	NS
23					
	Mean:	19.5	83		
	SD:	6.7	51		
	n:	16	16	.021	NS
20					
	Mean:	22.0	109		
	SD:	5.0	73		
	n:	10	10	.642	<.05
All Temps 1 WAY AOV		<.001	NS	0.352	<.001

FIGURE 49

THE RELATIONSHIP BETWEEN THE BLEEDING TIME AND THE TOTAL HEMOGLOBIN ON THE FILTER PAPER

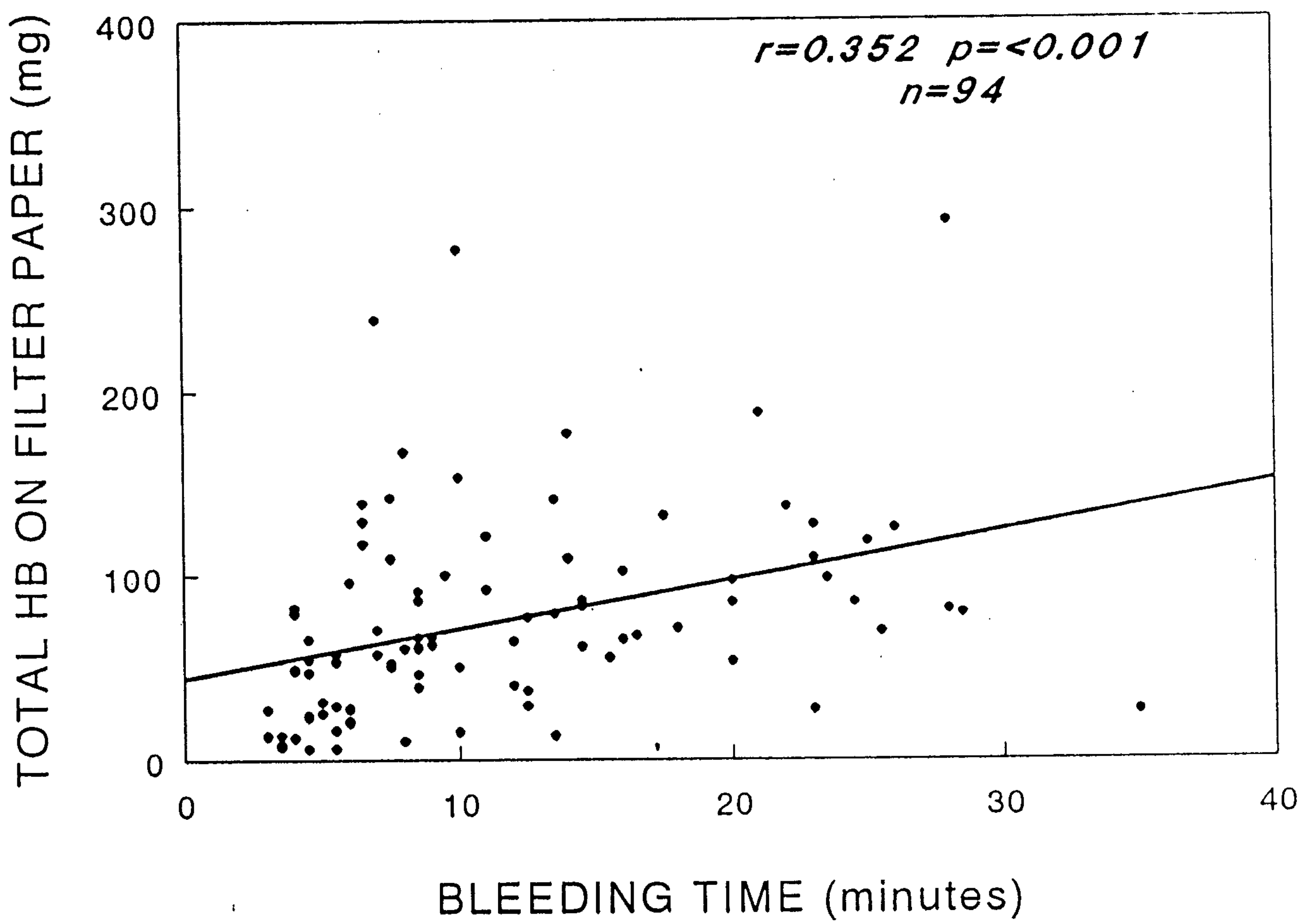

TABLE 17B

NATURAL LOGARITHM OF THE BLEEDING TIME AND THE NATURAL LOGARITHM OF THE TOTAL HEMOGLOBIN IN THE SHED BLOOD COLLECTED ON THE FILTER PAPER IN 16 NORMAL VOLUNTEERS AT SEVEN SKIN TEMPERATURES

Temperature (C)		Bleeding Time (min)	Total Hemoglobin Collected	Correlation Coefficient R	P
38					
	Mean:	1.62	4.11	.734	<.01
	SD:	.3	.9		
35 (n=10)					
	Mean:	1.62	3.84	.841	<.01
	SD:	.3	.8		
32					
	Mean:	1.83	3.44	.901	<.001
	SD:	.3	.9		
29					
	Mean:	2.32	4.15	.292	NS
	SD:	.3	.5		
26 (n=10)					
	Mean:	2.44	4.04	.801	<.01
	SD:	.3	.9		
23					
	Mean:	2.92	4.15	.311	NS
	SD:	.4	.9		
20 (n=10)					
	Mean:	3.09	4.48	.882	<.01
	SD:	.3	.8		
All Temps 1 Way AOV:		<.001	NS	.493	<.001

FIGURE 50

THE NATURAL LOGARITHM OF THE BLEEDING TIME AND THE NATURAL LOGARITHM OF THE TOTAL HEMOGLOBIN ON THE FILTER PAPER

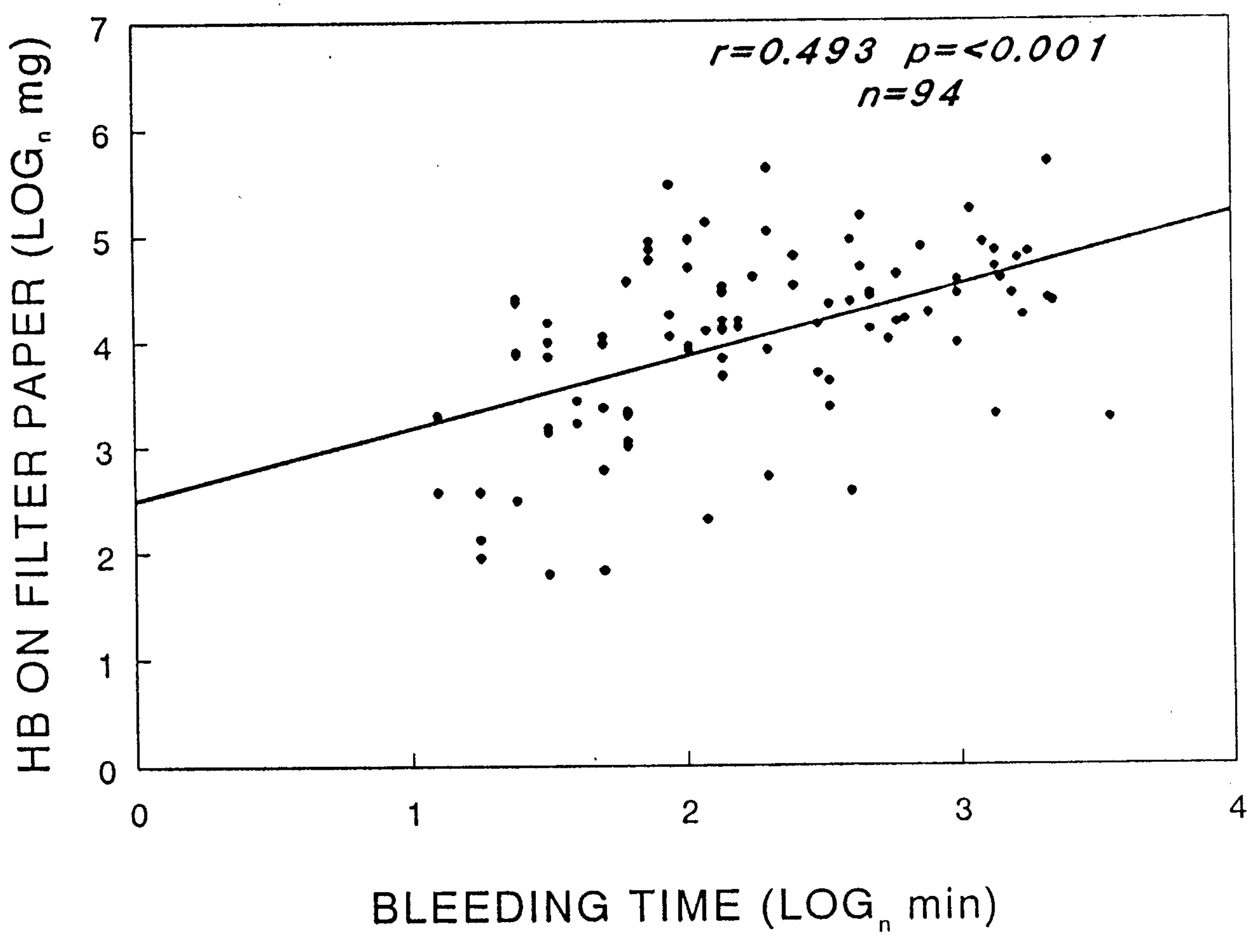

TABLE 18A

DIGITIZED AGGREGATION PATTERNS FOLLOWING STIMULATION OF PLATELET RICH PLASMA OBTAINED FROM THE ANTECUBITAL VEIN OF THE FOREARM SUBJECTED TO LOCAL WARMING AND COOLING TO ACHIEVE TEMPERATURES OF 37C, 32C, 28C, AND 22C WITH A COMBINATION OF AA AND ADP OR WITH RISTOCETIN ALONE WHEN THE PLATELET RICH PLASMA WAS MAINTAINED AT 37C

Temp	Aggregation to AA & ADP (digitized units/5 min) 0.05 mg/ml) (0.01 mM)	Ristocetin (digitized units/5min) (1.25 mg/ml)	Paired t Between AA & ADP and Ristocetin
32C			
Mean:	273	244	NS
SD:	61	102	
n:	16	10	
37C			
Mean:	283	242	NS
SD:	68	109	
n:	16	11	
28C			
Mean:	251	283	NS
SD:	67	57	
n:	16	10	
22C			
Mean:	261	234	NS
SD:	89	99	
n:	16	10	
1 Way ANOVA: NS	NS	NS	

TABLE 18B

THE NATURAL LOGARITHM OF THE DIGITIZED AGGREGATION PATTERNS FOLLOWING STIMULATION OF PLATELET RICH PLASMA OBTAINED FROM THE ANTECUBITAL VEIN OF THE FOREARM SUBJECTED TO LOCAL WARMING AND COOLING TO ACHIEVE TEMPERATURES OF 37C, 32C, 28C, AND 22C WITH A COMBINATION OF AA AND ADP OR WITH RISTOCETIN ALONE WHEN THE PLATELET RICH PLASMA WAS MAINTAINED AT 37C

Temp	Aggregation to AA and ADP (digitized units/5 min) (0.05 mg/ml) (0.01 mM)	Ristocetin (digitized units/5min) (1.25 mg/ml)	Paired t Between AA & ADP and Ristocetin
32C			
Mean:	5.58	5.39	NS
SD:	.3	.5	
n:	16	10	
37C			
Mean:	5.62	5.37	NS
SD:	.2	.6	
n:	16	11	
28C			
Mean:	5.48	5.62	NS
SD:	.3	.2	
n:	16	10	
22C			
Mean:	5.53	5.31	NS
SD:	.3	.7	
n:	16	10	
1 Way ANOVA:	NS	NS	

TABLE 19A

THROMBOXANE B_2 LEVELS FOLLOWING STIMULATION OF PLATELET RICH PLASMA OBTAINED FROM THE ANTECUBITAL VEIN OF THE FOREARM SUBJECTED TO LOCAL WARMING AND COOLING TO ACHIEVE TEMPERATURES OF +37C, +32C, +28C, AND +22C WITH A COMBINATION OF AA AND ADP OR WITH RISTOCETIN ALONE WHEN THE PLATELET RICH PLASMA WAS MAINTAINED AT 37C

Temp	AA (0.05 mg/ml) & ADP (0.01 mM) TxB2 Production Per Plt X10-5 (pg/0.1 ml)	Ristocetin (1.25 mg/ml) TxB2 Production Per Plt X10-5 (pg/0.1 ml)	Non Paired t Test Between AA &ADP and Ristocetin
32C			
Mean:	3.2	.8	<.01
SD:	1.2	.8	
n:	6	5	
37C			
Mean:	2.9	1.0	<.05
SD:	1.4	1.0	
n:	7	8	
28C			
Mean:	3.4	0.7	<.01
SD:	1.8	.6	
n:	7	6	
22C			
Mean:	3.12	1.1	<.05
SD:	2.0	.7	
n:	8	5	
1 way ANOVA:	NS	NS	

TABLE 19B

THE NATURAL LOGARITHM OF THE THROMBOXANE B_2 LEVELS FOLLOWING STIMULATION OF PLATELET RICH PLASMA OBTAINED FROM THE ANTECUBITAL VEIN OF THE FOREARM SUBJECTED TO LOCAL WARMING AND COOLING TO ACHIEVE TEMPERATURES OF +37C, +32C, +28C, AND +22C WITH A COMBINATION OF AA AND ADP OR WITH RISTOCETIN ALONE WHEN THE PLATELET RICH PLASMA WAS MAINTAINED AT 37C

Temp	AA (0.05 mg/ml) & ADP (0.01 mM) TxB2 Production Per Plt X10-5 (pg/0.1 ml)	Ristocetin (1.25 mg/ml) TxB2 Production Per Plt X10-5 (pg/0.1 ml)	Non Paired t Test Between AA &ADP and Ristocetin
32C			
Mean:	1.1	-1.3	<.05
SD:	.4	1.8	
n:	6	5	
37C			
Mean:	.95	-.7	<.01
SD:	.5	1.3	
n:	7	8	
28C			
Mean:	1.09	-1.14	<.05
SD:	.5	1.6	
n:	7	6	
22C			
Mean:	.82	-.39	NS
SD:	1.0	1.3	
n:	8	5	
1 way ANOVA:	NS	NS	

TABLE 20A

DIGITIZED AGGREGATION PATTERNS AND IN VITRO THROMBOXANE B_2 PRODUCTION BY PLATELETS IN RESPONSE TO RISTOCETIN ALONE OR A COMBINATION OF ARACHADONIC ACID AND ADP AT 22C AND 37C IN PLATELET RICH PLASMA SEPARATED FROM WHOLE BLOOD COLLECTED IN SODIUM CITRATE

N=5

FINAL CONCENTRATION OF ARACHADONIC ACID (.05 MG/ML) AND ADP (.01 mM)

	Aggregation (digitized units/5min)		Paired T 22-37	TXB2 Prod per per Plt ($x10^{-5}$)		Paired t 22-37
	22C	37C		22C	37C	
Mean:	237	265	NS	4.4	8.0	NS
SD:	103	58		6.0	9.3	

FINAL CONCENTRATION OF RISTOCETIN (1.25 MG/ML)

	22C	37C	Paired T 22-37	22C	37C	Paired t 22-37
Mean:	147	219		.07	.99	NS
SD:	53	96	NS	.07	.96	
Paired T Dual-Rist:	<.05	NS		NS	NS	

TABLE 20B

NATURAL LOGARITHM OF THE DIGITIZED AGGREGATION PATTERNS AND THROMBOXANE B_2 PRODUCTION BY PLATELETS IN RESPONSE TO RISTOCETIN ALONE OR A COMBINATION OF ARACHADONIC ACID AND ADP AT 22C AND 37C IN VITRO IN PLATELET RICH PLASMA SEPARATED FROM WHOLE BLOOD COLLECTED IN SODIUM CITRATE

N=5

FINAL CONCENTRATION OF ARACHADONIC ACID (.05 MG/ML) AND ADP (.01 mM)

	Aggregation (Digitized Units/5min) 22C	37C	Paired T 22-37	TXB2 Prod per per Plt ($x10^{-5}$) 22C	37C	Paired 22-37
Mean:	5.4	5.6	NS	0.3	1.5	NS
SD:	.5	.2		1.9	1.2	

FINAL CONCENTRATION OF RISTOCETIN (1.25 MG/ML)

	22C	37C	Paired T 22-37	22C	37C	Paired 22-37
Mean:	4.9	5.3		-2.9	-.47	
SD:	.4	.5	NS	.9	1.2	<.01
Paired T Dual-Rist:	<.05	NS		NS	NS	

TABLE 21

THE MEASURED AND TEMPERATURE CORRECTED BLEEDING TIME IN EACH OF TEN NORMAL VOLUNTEERS

SKIN TEMP:	38C		35C		32C		29C		26C		23C		20C	
	Meas	Corr	Meas	Corr	Meas	Corr	Meas	Corr	Meas	Corr	Meas	Corr	Meas	Corr
DONOR #														
1	3.5	3.5	3.5	3.5	3.5	3.0	5.5	3.9	5.5	3.0	8.0	3.2	10.0	2.5
2	3.0	3.0	3.0	3.0	4.0	3.4	8.0	5.6	8.5	4.7	16.0	6.4	20.0	5.0
3	4.5	4.5	4.5	4.5	6.0	5.1	10.0	7.0	12.0	6.6	15.5	6.2	23.5	5.9
4	7.0	7.0	7.5	7.5	8.5	7.2	11.0	7.7	12.5	6.9	22.3	8.9	19.8	4.9
5	6.0	6.0	5.3	5.3	6.0	5.1	9.0	6.3	14.0	7.7	17.5	7.0	25.0	6.3
6	8.0	8.0	7.5	7.5	7.5	6.4	12.0	8.4	12.5	6.9	13.5	5.4	23.0	5.8
7	7.0	7.0	6.5	6.5	9.5	8.0	11.0	7.7	14.5	8.0	23.0	9.2	24.5	6.1
8	7.5	7.5	6.5	6.5	10.0	8.5	10.0	7.0	14.0	7.7	21.0	8.4	28.0	7.0
9	4.0	4.0	4.0	4.0	6.0	5.1	8.5	6.0	8.5	4.7	16.0	6.4	28.0	7.0
10	4.0	4.0	4.5	4.5	4.5	3.8	8.5	6.0	18.0	9.9	20.0	8.0	20.0	5.0
Mean:	5.5	5.5	5.3	5.3	6.6	5.6	9.4	6.6	12.0	6.6	17.3	6.9	22.2	5.6
SD:	1.8	1.8	1.6	1.6	2.3	1.9	1.9	1.3	3.6	2.0	4.5	1.8	5.2	1.3
n:	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Range:	3.0-8.0	3.0-8.0	3.0-7.5	3.0-7.5	3.0-8.0	3.0-8.5	5.5-12.0	3.9-8.4	5.5-18.0	3.0-9.9	8.0-23.0	3.2-9.2	10.0-28.0	2.5-7.0

FIGURE 51

THE MEASURED AND CORRECTED BLEEDING TIME IN THE TEN (10) NORMAL VOLUNTEERS AT THE SEVEN (7) SKIN TEMPERATURES.