Two Patterns of Blood Flow Increase during Muscle Pump Action Produced by FM-Stimulation

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Two patterns of Blood Flow Increase during Muscle Pump Action Produced by FM-Stimulation

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key words : sinusoidal muscle exercise, hemoconcentration, dual blood flow, rabbit hindlimbs

Introduction

The causes of the increase in muscle blood flow at exercise is attributed, for one, to the enhancement of systemic circulation, centering on the heart, but the other is due to the hyperfunction of muscular microcirculation.[5)14)16)18)] The exercise hyperemia in the latter is further accelerated by the muscle pump.[1)4)26)28)]

The function of this muscle pump is considered to be still elevated structurally by the increasing size of capacitance vessels and its valves mainly in the line of the venule to the vein, dilating arterioles and increasing numbers of capillary in parallel line.[5)11)] Functionally, on the other hand, this may be caused largely by the increase in the difference of pressures between arterioles and venules, and by the dynamic rhythms of repeated contactions and relaxation of musle fibers surrounding the micro-vascular beds.[11)12)] Although the inner pressure and blood flow in micro-vessels have already been determined, the nature of the muscle pump has not been analyzed in a model from a viewpoint of exercise modes.

In the present study, observation is made first that the increase in blood flow by the muscle pump appears in accordance with various kinds of induced muscle exercise by the FM-stimulation method, simultaneously trying to record the functional dilating phenomenon of the microvascular system. The frequency modulating stimulations (FM-stimulations) with the function generator have been developed for the first time in order to analyze various kinds of induced muscle exercises and vascular reactions in set.

Methods

1. Experimental Animals and Preparations of Hindlimbs

Adult rabbits of both sexes weighing 2.5 to 4 kg were used for the experiment. The animals were fixed in supine position under urethane anesthesia (1g/kg of body weight). After the tracheal cannula was inserted, the femoral artery and vein of both hindlimbs were exposed, and then the right hindlimb was chosen for the determination, enabling it to stimulate the cut-end of the sciatic nerve. After finishing the surgical procedures, 300-1,000 unit/kg of sodium heparin was infused, and the concent-

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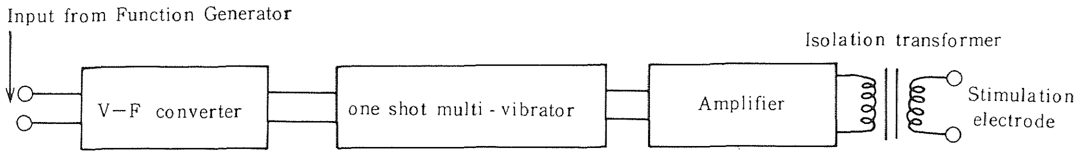

Fig. 1 The block diagram of the frequency modulating stimulator (FM-stimulator).

ration was kept approximately constant by supplementing it about an hour's interval.

2. Measurements of Muscle Blood Flow and Blood Pressure

Following administration of heparin, a polyethylene catheter was inserted into left femoral artery for recording arterial pressure by strain gauge. The right femoral artery remained intact. Arterial input pressure to the muscle was therefore the systemic one. The right femoral venous blood flow measured by the pulses with a photo cell drop counter were recorded with a pen as well as an output power with an interval-voltage converter. Calibration of blood flow was made by synchronizing a unit drop pulses with collected blood volume per minute, and were denoted in the minute blood flow per wet weight of the muscle (ml/100g/min). The blood in the reservoir was returned into the body of the animal through the left femoral vein by a small roller pump. The input blood pressure, respiratory phase and body temperature were all monitored so that the experimental conditions might be uniform.

The records including drop pulses, flow curve converted, blood pressure and respiration rate were made with polygraph (Nihon Kohden Co., Japan).

3. Analysis of Hematocrit, Total Plasma Protein, Plasma Crystalloid Osmolatity, Po_2, Pco_2 and pH

In the course of passing from the drop counter to the reservoir, blood was collected by a slender glass tube in oder to analyze the blood constituents. The measurement of hematocrit (Ht) was made by microcapillary method. The total plasma protein concentration (Protein) was analyzed with a D-Z type (II) refractometer (Kyoei Co., Japan) by cutting the capillary tube after determining the Ht at a portion containing the plasma.

The plasma crystalloid osmolality (osmolality) was analyzed with the semi-micro cryoscopic osmometer (H. Knauer & Co., West Germany) by collecting several capillary tubes after determining the Ht at a portions of the plasma. This method based on the fact that the osmolality of body fluids was proportionate with the freezing point of the solution, and the unit used was mOsm/kg・H_2O. The volume for a single determination was 0.1 to 0.2 ml.

Analysis of Po_2, Pco_2 and pH was made with the IL meter (IL Co., USA). The capillary tube for blood collection was 15 cm in length, collecting a little more volume for the sample than actually required, and analysis was made simultaneously with above-mentioned substances.

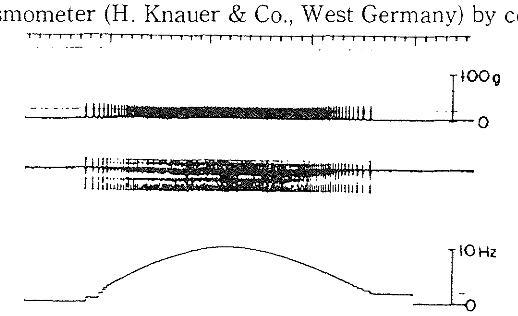

Fig. 2 The amplitude of tension force and its interval in accordance with sinusoidal FM-stimulation. Upper trace: tension force in M. tibialis anterior, Middle trace: pulses from the sinusoidal FM-stimulation, Lower trace: sinusoidal output curve from the D-A converter.

4. Evoked Muscle Exercises by FM-Stimulations

The FM-stimulation method is a new experimental method for the first time in the present trial. Fig. 1 shows the block diagram of the FM-stimulator. Various changes in voltage by the function generator (Wavetek Co., USA) underwent frequency convertions with the V-F converter, which reached the stimulating electrode after power amplification via the pulse generator (2 msec in width).

This apparatus was designed to exhibit the mode of exercise having a peak intensity, as observed in human foot movements of touching the ground while walking. In the present study a sinusoidal model of muscle exercise was induced by electric stimulation to the peripheral side of the cut-end of the sciatic nerve, and its intensity was obtained by continuously changing the frequencies between 0 -10 Hz for the nerve stimulations. The fixation of hindlimbs was made at two points, i. e. at the knee joint and at the end of the Os. tibia. The tension force of the M. tibialis anterior was recorded isometrically in vivo.

Results

1. Preliminary Experiments

The FM-electric stimulation method adopted in the present study, as compared with the conventional electric stimulation method with a constant stimulation rate, is so devised as frequencies should change during stimulation in accordance with the designated command signal, and the extent of muscle exercise varies as well. Fig. 2 showed an example, where the tachometric recordings of the changes in frequencies and frequency-responses of the tension force were shown, when the peripheral side of the cut-end of the sciatic nerve was stimulated in accordance with sinusoidal command signals. In this example, it was designed previously that the maximum frequency of 10 Hz might comes once in 30 seconds of the stimulation time. The tension force (upper trace) was obtained by connecting the cut-end of the tendon of the M. tibialis anterior to the force transducer with a wire. It was noted that the amplitude of the tension force, including this example, showed a decreasing tendency with the increase in frequencies.

2. Regular Stimulation

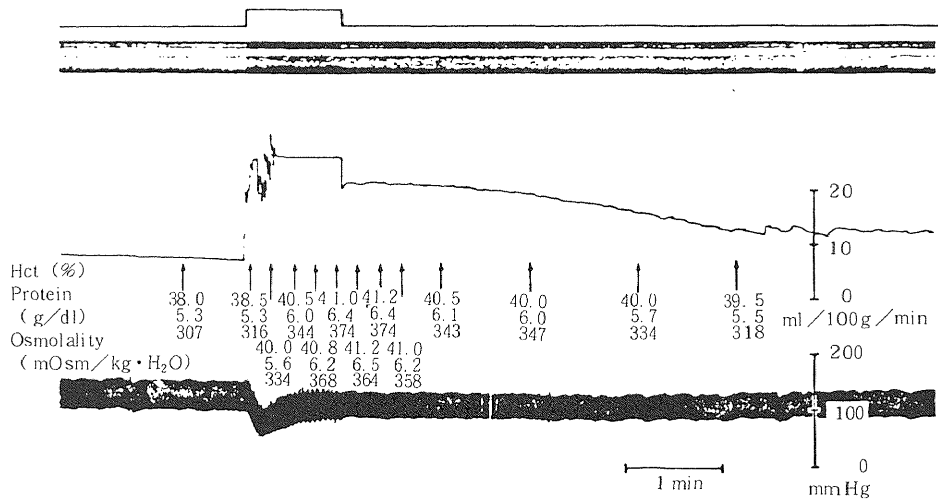

Fig. 3 The control record of muscle blood flow during expercise produced by 10Hz regular stimulation. Hematocrit (Ht), Total plasma protein (protein) and plasma crystalloid osmolality were determined begore, during and after the muscle exercise.

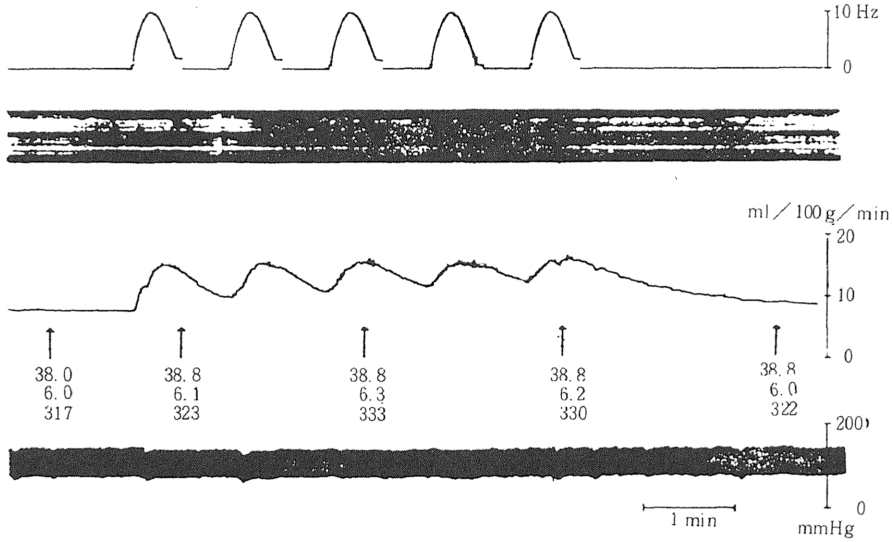

Fig. 4 The sinusoidal record of muscle blood flow during exercise produced by sinusoidal FM-stimulation. From upper to bottom: sinusoidal stimulations indicating 10Hz as the peak, Pulses from the sinusoidal FM-stimulation, sinusoidal flow curve via the D-A converter, and systemic blood pressure.

Fig. 3 showed the record of muscle exercise after conventional electric stimulation and the involved changes in blood flow. The intensity of exercise was 10 Hz for 1 minute.

The blood flow at rest indicated 10 ml/100g/min, while the blood flow after rapid muscle exercise recorded 2.5 times that of at rest, almost maintaining the level during exercise. Although an instantaneous decrease in blood in flow immediately after exercise was noted, the so-called post-exercise hyperemia continued thereafter for a few minutes. These hyperemic patterns with the increase in peak flow or the prolongation of the recovery time were in proportion to the intensity of muscle exercise. The Ht or protein showed an increase by 109% and 121%, respectively, and the osmolality, which can be a parameter of the tissue homeostasis, reached 122%.

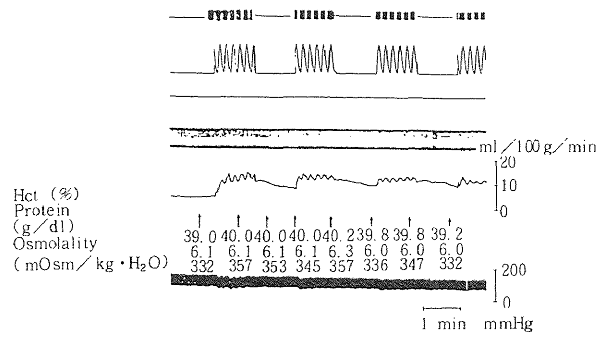

Fig. 5 Data showing the muscle blood flow during exercise produced by sinusoidal FM-stimulation. The peak of 10Hz is once in 10 seconds, with a resting phase for 1 minute after 6 times per minutes.

3. Sinusoidal FM-Stimulation

Fig. 4 showed the blood flow response induced by sinusoidal FM-stimulations, indicating an exercise for 30 seconds with 10 Hz as the peak, and a rest for 30 seconds, in a set. The blood flow level at rest, 8ml/100g/min, showed an increase whenever muscle exercise drew nearer to the intensity of 10 Hz (about 15ml), while it was decreased as it came nearer to the resting phase. These changes were in proportion with mechanical muscle exercise. The peak blood flow during sinusoidal exercise induced by FM-stimulation did not produce any more changes of the level of the peak flow, even the determinations were made repeatedly. On the other hand, the blood flow level at the resting phase which was provided for at each 30 seconds, showed a gradual increase with the progress of exercise. With our FM-stimulation method, the increase in blood flow at exercise and the increase in blood flow at rest were clearly distinguishable. The levels of the Ht, protein and osmolality determined simultaneously, were 102%, 105% ana 105% maximum, respectively, indicating a slight increase in each.

Fig. 5 showed an example where a sinusoidal exercise has the peak of 10 Hz once in 10 seconds, with a resting phase for 1 minute. As in this case where the frequencies of the peak were increased, an similarity was noted between the peak of muscle exercise and the peak of muscle blood flow, and

Table 1 The changes of muscle blood flow, hematocrit, total plasma protein, plasma crystalloid osmolality, PO_2, PCO_2 and pH before, during and after the sinusoidal muscle exercise.

	Pressure (mmHg)	B. Flow (ml/100g/min)	HT (%)	Protein (g/dl)	Osmol (mOsm/kg・H_2O)	PO_2 (mmHg)	PCO_2 (mmHg)	pH
control	107.6±3.64 (5)	8.2±0.7 (5)	42.7±0.90 (6)	5.4±0.2 (6)	341.2±8.95 (6)	47.5±2.6 (5)	31.4±0.5 (5)	7.380±0.02 (5)
Exercise 3′	106.4±2.95 (5)	17.8±0.9 (5)	43.2±1.66 (5)	5.8±0.3 (6)	337.8±4.41 (5)	36.1±2.3 (5)	44.4±3.4 (5)	7.269±0.03 (5)
5′	105.5±2.08 (5)	18.4±1.1 (5)	43.4±1.33 (6)	5.8±0.3 (6)	353.0±8.14 (6)	35.9±3.2 (5)	49.5±4.8 (5)	7.192±0.04 (5)
9′	103.7±2.74 (5)	17.8±1.6 (5)	43.4±1.40 (6)	5.7±0.2 (6)	356.8±13.7 (5)	34.2±3.9 (5)	46.0±6.4 (5)	7.189±0.05 (5)
15′	99.2±9.04 (5)	14.8±2.9 (5)	42.9±1.52 (5)	5.7±0.3 (6)	346.7±14.1 (6)	34.8±4.9 (5)	47.0±5.5 (5)	7.198±0.06 (5)
20′	107.3±2.71 (4)	18.2±1.3 (4)	42.3±1.54 (5)	5.5±0.3 (5)	336.3±3.60 (4)	38.9±2.9 (4)	42.5±3.8 (4)	7.255±0.03 (4)
26′	105.8±1.66 (4)	18.4±1.3 (4)	41.8±1.47 (5)	5.5±0.3 (5)	339.2±6.50 (5)	39.3±3.8 (4)	43.0±2.8 (3)	7.297±0.02 (4)
30′	108.3±1.53 (4)	18.5±1.7 (4)	41.7±1.64 (5)	5.4±0.2 (5)	340.2±8.22 (5)	37.1±3.7 (4)	42.7±1.8 (4)	7.278±0.02 (4)
Recovery 5′	104.9±3.40 (4)	11.1±2.8 (4)	40.3±1.24 (5)	5.2±0.3 (5)	332.8±7.39 (5)	43.7±3.7 (4)	36.1±1.5 (4)	7.355±0.02 (4)
10′ (Minutes)	101.8±2.85 (4)	9.1±0.6 (4)	40.6±1.34 (5)	5.2±0.2 (5)	345.0±7.27 (5)	49.2±5.1 (4)	28.8±3.5 (4)	7.383±0.01 (4)

(Mean ± S.E., n)

it could be observed that the level of blood flow at the time of low muscular activity after the peaks was considerably enhanced. It was also noted that the changes in the blood consituents were not so marked in such an long intervals exercise.

Table 1 shows the results of the sinusoidal muscle exercise for 30 minutes, and the changes in blood flow and compositions in blood observed at the recovery phase for 10 minutes, denoted in the mean values. The input blood pressure for 30 minutes was relatively stable, and the muscle blood flow at the time showed a value about twice as high as the value of the control. Considering the extent of the decrease in Po_2, the increase in Pco_2 and the decrease in pH, in the outflowing venous blood, this sinusoidal muscle exercise did not seem a heavy load, so that cach value of the Ht, protein and osmolatity also showed only a slight increase. However, the flow increased during 30 min exercise tended to maintain the level, while the Ht, protein and osmolality showed a decreasing tendency with the peak of the about 5-min level.

Discussion

In the present paper, it was designed that the muscular pumping action and microcirculatory responses can be revealed by chronologically changing the intensity of muscle exercise induced by stimulation, and for this reason the FM-stimulating apparatus was developed. As a result, observation of the changes in blood flow in response to chronologically changing exercise intensity became possible. In other words, it was made possible to observe two patterns of blood flow separately in the present experiment : one of them was the exhibition of the blood flow pattern agreeing with induced muscular exercise, and the other, a gradually increasing patten of blood flow with the progress of exercise. These two pattens of blood flow cannot be distinguished with regular stimulating as shown in Fig.3, which however can be clearly manifested with sinusoidal stimulation shown in Fig. 4 and 5, even with an exercise of the same intensity of 10 Hz.

Muscle blood flow at exercise is increased further by the rhythmic contraction of mucle ; i. e. muscle pump.[3)11)12)] The fundamental constructive elements of the muscle pump is the existence of venous valves and the decrease of pressure in capacitance vessels.[11)] It can be considered that the degree of dynamic actions of contractions and relaxations of muscle fibers may influence on the capacities of the microvascular system including the responses of asterioles. Although the dilative effect of muscle vibration on vascular smooth muscle is known,[29)] the degree of the mechanical vibrations hyperemia is not so large, as compared with the exercise one.[19)]

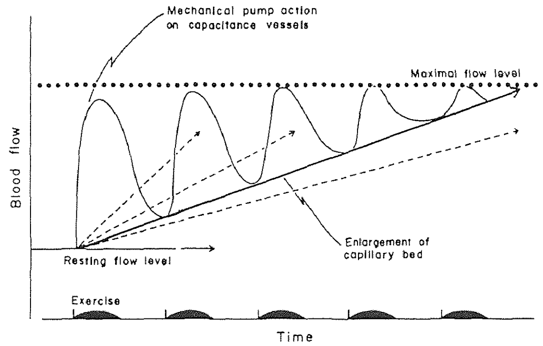

Fig. 6 Schema representing the hypothesis on the activities of dual blood flow channels produced by sinusoidal FM-stimulation.

From the experiments in Fig. 4 and the rewritten schema as shown in Fig. 6, it is believed that the blood flow pattern in response to the sinusoidal exercise may perhaps be mechanical pump actions affecting capacitance vessels. Further, the blood flow pattern increasing gradually during the resting phase may indicate the functional dilation of the microvascular system including the dilation of arterioles and precapillary sphincters as well as the reopening of closed capillaries. In the graph in Fig. 6 the dotted line shifting to the left above indicates higher peak frequencies per unit time, even in the sinusoidal exercise of the same 10 Hz-peak, whereas the dotted line in the lower part in the right shows a case of slow peak frequencies.

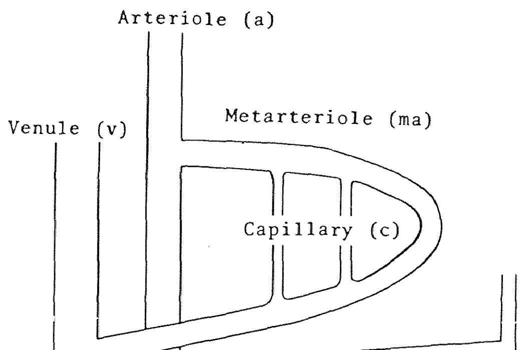

Fig. 7 Schema representing the simple structure of dual blood flow system and lymphatics. (from Nagashima, Ch.).

Intensities in regular and/or sinusoidal exercise can be discussed in terms of the changes in blood compositions : the fact that the Hb, Ht and protein are increased during exercise, regardless of systemic or local exercise is known, [2][4][24][25][34][35] and these elements have been made the indices of hemoconcentrations.[6][22][35][37][39][40] In the present experiment, the increase in the Ht or protein was noted more markedly in regular exercise than in sinusoidal exercise, and the increase in osmolality[30][31] as well was observed more markedly in regular exercise. It can be understood that the increase in the Ht is considerably high, even without correcting the decrease in the volume of blood corpuscles due to the temporal increase in the crystalloid osmolality in plasma, and that the increase in Ht and protein is an evidence of the exhibition of hemoconcentrations.[16][35] Since this plasma fluid filtration is performed only through the capillaries, it may be considered that a number of capillary channels mightfunction immediately after the start of regular exercise, and the shift of the fluids might take place. This is the reason for the following initiation of lymph channels activity in an in-vivo experiment.[9][21][42] In sinusoidal exercise, on the other hand, it seems that a gradual incerase in capillary channels may appear, without the occurrence of marked hemoconcentrations from the beginning (Fig. 4, Table 1).

In 1949, Zweifach showed a typical structure of microvessel in skeletal muscle, which is simplified in Fig. 7 (from Nagashima, Ch). The model consists of the metarteriolar channel of fast flow and the ture capillary channel of slow flow. The authors consider that the selective manifestation of these dual blood flow channels might be observed, depending on the mode of muscular exercise.

According to Folkow & Neil[11], the phenomenon of the muscle pump is attributed in essence to the generated pressure difference by muscle activities within capacitance vessels, centering on venous valves, and therefore the decrease in the inner pressure in veins due to muscular compression and relaxation is emphasized. Generally, the function of the muscle pump depends on the mechanical actions of muscle compression and on the capacities of the vascular network. In the former, the number of muscle fibers participating in muscle contraction and muscular compression frequencies are associated, while in the latter, the venous tone and the inflowing blood volume into capacitance vessels are related.

During muscle exercise, the dilation of arterioles and precapillary sphincters take place, added with the increase in active capillaries.[8][17][31][36] This is for these reasons that the increase in parallel flow channels of capillaries induces the decrease in peripheral vascular resistance, which supplies ample blood flow to the peripheral tissue, and simultaneously it must increase the perfused flow into venules with proper exercise.

The increase in the venous return from the microcirculatory system leads to the increase in the cardiac output in the systemic circulation, thus playing an important role in the redistribution of blood to the microcirculatory system.[23][32] Although the alternative control mechanism in dual blood flow channels[20] is not entirely clear, it is vital for local homeostasis as well as for venous return, possibly regulating the long-lasting performance of the muscle.[33]

As the results of the present experiment show, when severe isometric exercise continued, hemoconcentrations, i. e. plasma fluid filtrations, occur rapidly, though the increase in number of capillaries is observed. The outflow of K^+ in intercellular fluids causes electrolytic environments of muscle fibers to be abnormal. It is considered that the elevation of osmolality in blood caused K^+ and metabolites induced the dilation of vascular smooth muscles,[7][10][27][30][31][38] but the local failure of homeostasis might eventually reduce the prolongation of muscle exercise.

The significance of the dual blood flow channels may be to act alternatively with each other within an extent not to cause in extreme failure of homeostasis, which will maintain a well-balanced microcirculation. It is therfore considered that the achievement of such prolonged muscle exercise may require a biomechanical technique based on the dynamic characteristics of microcirculation.

Summary

The aim of this study is to analyze the relation between the mechanical elements of the muscle pump and the physiological elements of vascular dilation with our newly developed FM-stimulation method, and the authors analyzed various induced muscle exercise in the hindlimbs of rabbits under urethane anesthesia, as well as the responses in blood flow and the changes in blood constituents in them, and the following results were obtained :

1). The FM-stimulation method is the one using a stimulator changing the frequencies within a range of 0 to 10 Hz, which sets artificially changeable stimulating situations of nerve impulses to the target organ.

2) When the muscle blood flow is increased due to sinusoidal stimulations, two patterns appeared ; a blood flow increasing with mechanical muscle movements as well as a blood flow gradually increasing with the progress of the times of exercise were observed.

3) It was noted by the analysis of the components in blood (Ht, protein and osmolality) that even in the exercise of the same intensity of 10 Hz, local circulatory load was milder in sinusoidal muscle exercise than a mere isometric exercise.

These results appear to reveal the existence of alternative coupling of fast flow channel and slow flow channel during muscle exercise, centering on muscle endurance and local homeostasis.

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和 文 要 約

本研究では，筋ポンプのメカニカル要素と血管拡張の生理的要素の関係を新しく開発したFM刺激法で分析することをねらいとし，ウレタン麻酔下のウサギ後肢筋の各種の誘発筋運動と血流応答，血中組成分析を行った。これは持久性運動の基礎である筋血流を，運動様式と微小循環反応の組合わせから追求しようとしたものである。人体の研究では総合的な血流の記録だけで限界があり，持久力の向上や対策のための血流維持条件の研究には実験研究が必須であると考えるものである。実験結果は以下に示す通りであった。

1） FM刺激法は周波数を0～10 Hzの範囲で変動させる刺激装置を用いた方法であり，神経impulseのtarget organに対する可変的な刺激状況を人工的にセットしたものである。

2） sinusoidal stmulationによる筋血流の増大現象の際には2つのパターンが発現し，メカニカルな筋運動と共に増大する血流と運動回数の進展と共に徐々に増大する血流とが観察された。

3） 血中組成（Hct, protein, osmolality）の分析より，同じ10 Hz程度の運動であっても，インターバルを含めた筋運動では，単なるisometric exerciseより局所の循環負担が軽微であった。

以上の結果より，二相血流路のひとつであるfast flow channelを筋活動と同期して観察し，同時に経時的な記録を通じてslow flow channelの存在を確認した。この二相性血流路は，筋ポンプの性能に関係する要素であり，運動時ばかりでなく回復期の筋組織における血流効率を左右する重要なはたらきをするものと考えている。要はこの両channelsが局所の処理とvenovs returnにバランスよく役立つことであり（homeostasis），その均衡維持を運動型式の側から追求し，負債因子の低減とperformanceの向上にむけた研究にしたいと念願している。