DYNAMIC VISCO-ELASTIC PROPERTIES OF GLYCEROL-EXTRACTED MUSCLE FIBERS

Moto Matsumura* AND Torao Nagai**

Department of Physiology, Sapporo Medical College, Sapporo

Szent-Györgyi (1949) demonstrated that the maximal tension produced by glycerol-extracted rabbit psoas muscle fibers was essentially equivalent to that of living muscle. It has also been reported that the other mechanical features of the muscle model, for example, maximal shortening, speed of shortening, temperature dependence of tension development, elastic properties, mechanical efficiency and so on are quantitatively similar to those of living muscle (Weber and Portzelh, 1954).

On the other hand, many attempts to analyse the mechanical properties of the living muscle have lead to the conclusion that the muscle fiber behaves like high elastic substances or some types of visco-elastic models (Levin and Wyman; Winton, 1936; Hill, 1951; Reichel, 1952). Buchthal and Kaiser (1951) introduced the concepts of rheology into the muscle physiology, and recently, Machin et al. (1959, 1960, 1962) have succeeded in analysing the mechanical properties of the beetle muscle into viscous and elastic elements.

The purpose of this work is to obtain the detailed information on the visco-elastic properties of glycerol-extracted muscle fibers during contraction and relaxation, and thereby to find out some clue to the mode of contractile system.

METHODS

The glycerol-extracted muscle fibers were prepared after Szent-Györgyi's method (1949). Psoas muscle fibers of rabbits were used, which had been preserved at their length in situ in 50 per cent glycerol at −5°C for about three months. The diameter of fine bundle of fibers was around 0.4 mm and the length around 30 mm.

Adenosinetriphosphate (ATP) employed was obtained from Sigma Chemical Company. The composition of reaction mixture was as follows: KCl 100 mM, MgCl₂ 2 mM, tris-acetate buffer (pH 6.8 or 7.0) 60 mM and ATP 2 mM. All the experiments were performed at room temperature, between 19°C and 25°C.

Received for publication January 30, 1963.

* Visiting research associate. Present address: Department of Physiology, School of Medicine, Juntendo University, Hongo, Tokyo.
Both sinusoidal and transient methods were applied to this study. The principles of analyses of both types were explained in detail by Machin and Pringle (1960). Main experimental equipment is illustrated in Fig. 1. The muscle fibers (m) were laid horizontally in the bath of 15 ml in volume. One end of fibers was tied by a thread to the vibrator (V) and the other end to the mechano-electric transducer (RCA 5734) (T) which is fixed to the micromanipulator (M). The vibrator was a common ink writing penmotor, and it was set so as to move in the longitudinal direction of muscle fibers. Its resonant frequency was 50 c/sec. The output of the AC oscillator (AC) was adjusted so that the amplitude of vibration could be kept constant in the range of frequencies between 20 c/sec and 200 c/sec. The highest frequency was limited by the mechanical input impedance of the transducer, and the lowest was limited by the performance of the oscillator. Lower frequencies under 20 c/sec were generated by a mechanical device of a pulley similar to the method of Pieper et al. (1951). When the transient method was applied, the AC oscillator was replaced by a square pulse generator.

The sinusoidal change in length was measured by a phototube (P), and the sinusoidal change in tension by the RCA 5734. In order to complete the penetration of ATP into the fibers, the bundle should be as possible as thin (Hasse1bach, 1952). A light glass shaft of 8 mm in length was attached to the apex of the 5734 tube so that it might be sufficient to detect the small tension developed by a thin bundle of fibers. The resonant frequency of modified transducer was 1000 c/sec. The inertia of the moving coil of vibrator was fairly large. The force, exerted by an oscillator, necessary to move the vibrator was far larger than the force developed by muscle fibers, therefore the movement of the vibrator was not be affected whether muscle fibers were contracted or relaxed.
The terms measured were,

- muscle length (during preservation in glycerol solution or in situ): $L_0$ mm
- muscle length (in the solution containing ATP or other salts): $L$ mm
- contraction tension: $P$ g wt
- diameter of bundle of fibers: $D$ mm
- change in length (peak to peak): $\Delta L$ mm
- change in tension (peak to peak): $\Delta P$ g wt
- oscillation frequency: $f$ c/sec

RESULTS

1. Changes in stiffness of glycerol-extracted muscle during contraction and relaxation. The term of stiffness means the ratio of the change in tension to the change in length, ($\Delta P/\Delta L$), according to the Buchthal's definition (1944).

Fig. 2 shows the isometric contraction tension ($P$) superposed with the sinusoidal changes in tension ($\Delta P$), when the sinusoidal changes in length ($\Delta L$) are imposed on the fiber during contraction and relaxation. As the muscle is held slackened a little, it shows free shortening at the beginning of contraction, no tension being developed. After having shortened to its fixed length, the muscle contracts under the isometric condition (Fig. 2, A). Sinusoidal change in tension ($\Delta P$) is small at first, and increases in parallel with $P$ as the contraction advances. After a minute or so both $P$ and $\Delta P$ reach to their peak (Fig. 2, B). Fig. 2 C, D and E show $P$ and $\Delta P$ during relaxation. Immediately after the ATP solution has been replaced with 100 mM pyrophosphate-Na, sudden drops in $P$ and $\Delta P$ are found, the latter being always more remarkable. The relaxation is not complete a few minutes after replacement the solution in the bath, and $\Delta P$ is preserved in small amplitude.

The relation between $\Delta P$ and $P$ is shown in Fig. 3. As $\Delta L$ is kept constant throughout the contraction cycle, $\Delta P$ may be regarded to represent the relative value of the stiffness. Therefore, Fig. 3 shows the stiffness-tension
During contraction stiffness increases at first more quickly than the tension develops, then in proportion to the tension and finally approaches to the stationary value before the tension reaches its peak. During relaxation stiffness decreases more suddenly at first than the tension falls, thereafter with linear relation to tension. In the range where stiffness-tension relation in contraction or relaxation is linear, its tangent in each process is nearly equal to each other.

![Diagram](image)

**Fig. 3.** Relations between stiffness and contraction tension during contraction and relaxation. The same example as in Fig. 2.

2. **Static and dynamic tension-length diagram.** A static tension-length diagram of fully contracted muscle immersed in reaction mixture is illustrated in Fig. 4, A. Determination of static diagram is very difficult, because the tension at a certain muscle length depends on the procedure, whether isometric or isotonic, and on the time used for determination. In this study, the fibers were stretched slowly by 2 mm in one step and the resulting tension was recorded 15 minutes after extension. From our results it may be regarded that the static tension-length relation is approximately linear.

In Fig. 4, B, the static diagram is compared with dynamic diagram at the length around 76.7% of L₀. In the static diagram the tension change (dP) against the length change (dL) of 10% of L₀ is 12.5% of P₀, while in the dynamic diagram dP against dL of 1% of L₀ is about 40% of P₀. Here, P₀ is the maximum contraction tension. Thus, the dynamic stiffness is 30 times larger than the static stiffness. Granting that the dynamic stiffness is the sum of the elastic and viscous stiffness while the static stiffness represents the elastic stiffness only, the striking difference between the dynamic and the static stiffness can not be completely explained. Perhaps the muscle would suffer a plastic deformation by 2 mm stretch during the determination of the static diagram.
The sinusoidal analysis is to determine the relationship between the amplitudes or the time course of $\Delta P$ and of $\Delta L$ shown in Fig. 4, B.

3. The limit of amplitude of $\Delta L$ and the yielding area. Provided $\Delta L$ is less than 1.5% of $L_o$, $\Delta P$ is in a direct proportion to $\Delta L$, and the fibers obey Hook's law. This proportionality is no longer maintained if $\Delta L$ is greater than 2% of $L_o$. The sinusoidal curves of tension are distorted in the half cycle of release by length changes of 3 or 4% of $L_o$, and the fibers are torn by a stretching 5% of $L_o$.

4. The difference of complex modulus between contracted and relaxed muscle fibers. The complex modulus is defined as $\Delta P \cdot \Delta L^{-1} \cdot A^{-1} \cdot L$. Here, $A$ is the cross sectional area of the bundle and can be calculated from its diameter or its weight and length. The complex modulus is the sum of viscous and elastic modulus, and is called the visco-elastic modulus. The complex modulus of contracted muscle measured at the frequency of 20 or 40 c/sec and at the length of 80% of $L_o$ is 7000 or 10000 g wt-cm$^{-2}$. A few examples are shown in Fig. 5 and Table 1. The relaxed muscle, on the other hand, at the length of 120% of $L_o$ has the value of 800 or 1200 g wt-cm$^{-2}$ when the relaxation is performed in...
100 mM pyrophosphate-Na solution or 1 M KCl solution, and about 2000 g wt·cm⁻² in 50 mM pyrophosphate-Na solution containing 2 mM MgCl₂. The complex modulus of relaxed muscle is always smaller than that of contracted one, though the amount of the difference between the two depends upon the concentration of salts.

5. Elastic modulus and viscosity of the muscle fibers. The elastic modulus and viscosity are calculated from the amplitudes and the phase difference of sinusoidal changes in length and in tension. The range of frequencies used here is between 20 and 200 c/sec. The experiments at lower frequencies (1 or 10 c/sec)

---

**Fig. 5.** Effects of oscillation frequencies on the complex modulus of contracted (A) and relaxed muscle (B) in 100 mM pyrophosphate-Na. \( L_0 = 29 \text{ mm}, \ L = 24 \text{ mm} \) in A and 31 mm in B. \( \Delta L = 0.7\% \ L_0, \) diameter of muscle bundle = 0.4 mm, muscle weight = 3 mg, 20°C.
do not offer any advantages for this study. As is described later, the complex modulus is dependent on the muscle length. In this section the elastic modulus and viscosity are determined at the length of about 80% of L₀ for contracted muscle and about 120% of L₀ for relaxed muscle.

First, the ratio of ΔP/ΔL and its frequency dependence were measured. Fig. 5 shows the complex moduli of the muscle fibers against the frequencies of vibration. The complex moduli (|Z|) are increased upwards concavely when higher frequencies are applied. As regards to the contracted muscle, the |Z| at the frequency of 200 c/sec is 1.8 times larger than that at 20 c/sec, while as to the relaxed muscle, the |Z| at 200 c/sec is 3 or 4 times larger than that at 20 c/sec. It may be said that the complex moduli of relaxed muscle are more dependent on frequencies than those of contracted muscle.

Next, the phase difference (φ) should be concerned. The change in ΔP always precedes to the change in ΔL and φ is always positive. In the case of the contracted muscle, φ is 20° at 20 c/sec, increases with the frequencies and is about 45° at 200 c/sec. On the other hand, in the case of relaxed muscle, the situation is somewhat different. The φ against frequencies shows the convex curve with the maximum value of 40° at 100 or 120 c/sec while 25° at 20 c/sec and 20° or 30° at 200 c/sec. The weight of muscle fibers used here was about 3 mg but it might be neglected in the solution. Elastic modulus (G), viscosity (R) are calculated from the equations (1) and (2), or (3) and (4). One example is shown in Table 1.

\[
|Z|^2 = G^2 + (2\pi fR)^2 \quad \text{(1)}
\]

\[
\tan \phi = \frac{2\pi fR}{G} \quad \text{(2)}
\]

\[
|Z|^2 = \frac{1}{G^2} + \frac{1}{(2\pi fR)^2} \quad \text{(3)}
\]

\[
\tan \phi = \frac{G}{2\pi fR} \quad \text{(4)}
\]

Here, equations (1) and (2) can be fitted to the Voigt model and equations (3) and (4) to the Maxwell model. If the frequencies of vibration are less than 40 c/sec, viscosity plays a small role and the complex modulus is determined mainly by the elastic part.

When the sinusoidal changes in tension are imposed on a visco-elastic material, it is more convenient to describe the Voigt model, while in the case of the sinusoidal changes in length it is described by the Maxwell model. When the complex modulus is divided into a real part (ΔL and ΔP are in phase) and an imaginary part (ΔL and ΔP are out phase), in Voigt model the real part is equal to G and the imaginary part is equal to 2πfR, and in Maxwell model the real part is equal to G·(2πfR)^2/(G^2+(2πfR)^2) and the imaginary part is equal to
TABLE 1.
Contracted muscle.

<table>
<thead>
<tr>
<th>frequencies c/sec</th>
<th>Voigt</th>
<th></th>
<th></th>
<th>Maxwell</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
<td>100</td>
<td>160</td>
<td>40</td>
<td>100</td>
<td>160</td>
</tr>
<tr>
<td>(</td>
<td>Z</td>
<td>)</td>
<td>7800</td>
<td>9000</td>
<td>10800</td>
<td>7800</td>
</tr>
<tr>
<td>(G)</td>
<td>7200</td>
<td>7800</td>
<td>8500</td>
<td>8200</td>
<td>10400</td>
<td>14000</td>
</tr>
<tr>
<td>(R)</td>
<td>12</td>
<td>7</td>
<td>7</td>
<td>80</td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td>(\tau)</td>
<td>1.7</td>
<td>0.9</td>
<td>0.8</td>
<td>9.5</td>
<td>2.7</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Relaxed muscle

<table>
<thead>
<tr>
<th></th>
<th>40</th>
<th>100</th>
<th>1330</th>
<th>40</th>
<th>100</th>
<th>1330</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(</td>
<td>Z</td>
<td>)</td>
<td>770</td>
<td>950</td>
<td>1330</td>
<td>770</td>
</tr>
<tr>
<td>(G)</td>
<td>650</td>
<td>750</td>
<td>*</td>
<td>900</td>
<td>1200</td>
<td>*</td>
</tr>
<tr>
<td>(R)</td>
<td>1.3</td>
<td>1.0</td>
<td>*</td>
<td>7.0</td>
<td>2.6</td>
<td>*</td>
</tr>
<tr>
<td>(\tau)</td>
<td>2.0</td>
<td>1.3</td>
<td>*</td>
<td>7.8</td>
<td>2.2</td>
<td>*</td>
</tr>
</tbody>
</table>

\(Z\) : complex modulus g wt·cm⁻², \(G\) : elastic modulus g wt·cm⁻², R : viscosity g wt·cm⁻²·sec. \(\tau\) is the retardation time or relaxation time and equal to \(R/G\), msec. 
*: difficult to be determined, see text in discussion.
Contracted muscle: at the length of 82% \(L_o\) in reaction mixture.
Relaxed muscle: at the length of 115% \(L_o\) in 100 mM pyrophosphate Na solution.

Fig. 6. Stiffness as a function of muscle length of contracted (A) and relaxed muscle (B). \(L_o=38\) mm in A and 26 mm in B. \(f=20\) c/sec, 23°C.
Accordingly, both models are mathematically equivalent to each other, though the elastic modulus and viscosity shown in TABLE 1 are different on the hypothetic model.

6. The effect of muscle length. The static stiffness is independent on muscle length, as the relation between $P$ and $L$ is linear as shown in FIG. 4, A. The dynamic stiffness, however, is always increased if the muscle is stretched (FIG. 6). The increase in stiffness is more remarkable in the relaxed muscle than in the contracted muscle.

7. Transient analysis. FIG. 7 shows the responses of contracted (A) and relaxed muscle (B) during and immediately after the sudden changes in length. It takes 8 msec to complete stretching, because of the compliance of the vibrator. The tension rises while the the extension is continued and reaches its maximum at the time when the extension has been completed. The damped oscillation with a period of 3 msec superposed on the tension curve represents the resonant oscillation of the recording system and muscle fibers.
The transient tension-length diagram is obtained by plotting the rise of tension against the amplitude of extension in the initial course. The transient diagram shows the similar features to the dynamic tension-length diagram obtained by the sinusoidal methods, concerning with the yielding area, the magnitude of stiffness and its difference between contracted and relaxed muscle.

The time course of decay of tension after quick stretch was followed for one second (Fig. 7, C and D). The tension declines suddenly in 30 or 50 msec and hereafter rather slowly, but never returns to the initial level within a second or so. The secondary course is exponential, and the time constant is 1.2 or 2.0 sec with the contracted muscle (Fig. 7, C) and about 10 sec with the relaxed muscle (Fig. 7, D).

DISCUSSION

As REICHEL (1958) pointed out, the visco-elastic properties of the muscle during contraction could be measured only when the change in length was applied so quickly that the contraction remained constant throughout. Such a condition would be always satisfied as far as glycerol-extracted fibers are concerned.

The glycerol-extracted muscle fibers are easily yielded by the change in length beyond 1.5% of L0. Its elastic limit is lower than that of living muscle. The difference would not be necessarily attributed to the properties of contractile substances but of sarcolemma, because the sarcolemma plays a considerable role in visco-elastic properties of living muscle (RAMSEY and STREET, 1940; BUCHTHAL and WEIS-FOGH, 1956).

REICHEL (1960) investigated the changes in dynamic stiffness during a single twitch of tortoise muscle. According to him, the ratio of stiffness to tension during contraction was consistent with the ratio during relaxation or passive stretch. The same method, as shown in Fig. 2, could be applied to the glycerol-extracted muscle. Although it takes some times for ATP or other salts to penetrate completely into the interior fibrils of glycerol-extracted muscle (HASSELBACH, 1952), the tangent of stiffness-tension curve during contraction or relaxation is almost equal to each other (Fig. 3). Similar to living muscle, the dynamic stiffness can be represented as a simple equation to contraction tension.

in contraction process,

\[(\text{stiffness}) = F_C(\text{tension}) + A_C \quad \ldots \quad (5)\]

in relaxation process,

\[(\text{stiffness}) = F_R(\text{tension}) + A_R \quad \ldots \quad (6)\]

$F_C$ is nearly equal to $F_R$, and $A_C$ is positive while $A_R$ is negative. It is also suggested that in the range of linear relation the relaxation may be in mole-
cular mechanism a reverse reaction to contraction.

As shown in Fig. 4, A, a large extent of stretch would cause the plastic deformation in the fibers. Weber (1959), excluding the plastic effect, determined the static modulus. He showed that the modulus was 5000 g wt·cm⁻² for contracted muscle and 250 g wt·cm⁻² for relaxed muscle, and that these values were equal to those of the living muscle. Similar results were also obtained by Bozler (1956). The static elastic modulus determined by them was about a half of our values upon the complex modulus (Fig. 5 and Table 1).

A little has been known on the viscosity of muscle fibers. In our laboratory, it has been obtained by the method of proper vibration (Takauiji, 1962) that the retardation time of relaxed glycerol-extracted muscle fiber was about 1 msec. Buchthal and Kaiser (1951) showed that the contracted frog muscle had the retardation time of 3 or 10 msec. Machin and Pringle (1960) analysed the elastic modulus and viscous modulus of resting beetle muscle and presented a model with a time constant of 1.2 msec. The viscosity and retardation time calculated by these authors are not widely different from ours. It must be remembered, however, that we neglected the mass of fibers. The phase difference is not in a simple relation to oscillation frequency in relaxed muscle fibers, and the damped oscillations with the period of 3 msec are observed after sudden stretch (Fig. 7). The mass seems not to be negligible especially in a relaxed muscle and in the range of high frequencies. Further investigations would be needed for the determination of the exact values of elastic modulus and viscosity.

The tension developed by quick stretch decays exponentially (Fig. 7, C, D), and the time constant of tension fall is 1.2 or 2.0 sec in a contracted muscle, and about 10 sec in a relaxed muscle. These facts indicate the muscle has a system with a large relaxation time in addition to the system obtained by sinusoidal analysis. Edman (1959) investigated the time course of tension fall after quick stretch in glycerol-extracted muscle, and showed that its half decay time was about 4 sec. Buchthal and Kaiser (1951) also showed two kinds of systems whose time constants were quite different from each other. Weber (after Reichel, 1952) suggested that the time course of length change after sudden loading would be of two types, namely, Momentandehnung und Nachdehnung, and each of them were carried out independently. Our results support his concepts. According to Mashima and Matsumura (1960) there are two processes or elements in a contractile system of frog skeletal muscle, one of which contracts strongly and quickly whereas the other rather weakly and slowly. It would be plausible that there are two kinds of contractile systems possessed of different visco-elastic properties.

The elastic modulus and viscosity measured here do not concern with the series elastic component only described by Hill (1953) and Jewell and Wilkie (1958). There would be no reasons to regard that the contractile component
of glycerol-extracted muscle in contracted or relaxed state might be a rigid body.

Muscle length is one factor which determines the stiffness. The stiffness is increased as the fibers are stretched. According to Buchthal et al. (1944), stiffness in the contractile substance is of two types, structural and chemical stiffness. It may be also possible to express them with the term of inter- and intramolecular stiffness (Nagai et al., 1955). Huxley and Peachey (1961) reported that the contraction can occur only if there was an overlap between actin and myosin filaments. The glycerol-extracted muscle is not broken until it is stretched over 160% of L₀, which corresponds to the limit of an overlap between two filaments. The increase in stiffness by extension of muscle fibers (Fig. 6) could not be explained from the assumption that the force exerted by fibers would be proportional to the area of an overlap. Perhaps, by extending fibers intramolecular stiffness would be changed.

From the investigation upon the elastic modulus, viscosity and their changes during contraction, it would be suggested that the contraction of glycerol-extracted muscle might be performed on the same mechanism as that of living muscle.

SUMMARY

1. The dynamic visco-elastic properties of glycerol-extracted muscle were investigated.
2. The dynamic stiffness increases during contraction and decreases during relaxation. The stiffness-tension relation is linear in most parts of either contraction or relaxation processes, and its tangent is almost equal to each other.
3. When the sinusoidal changes in length (peak to peak) are less than 1.5 percent of muscle length, the fiber's obey Hooke's law.
4. The complex modulus measured at the frequencies of 20 or 40 c/sec of contracted muscle is 7000 or 10000 g wt·cm⁻². The modulus is decreased to 800 or 1200 g wt·cm⁻² if the muscle is relaxed in 100 mM pyrophosphate-Na solution or 1 M KCl solution, and to 2000 g wt·cm⁻² in 50 mM pyrophosphate-Na solution containing 2 mM MgCl₂.
5. Elastic modulus and viscosity are calculated based on the rheological analysis. The retardation time is 0.8 or 2.0 msec.
6. When the muscle is stretched, the stiffness is always increased.
7. From the time course of decay of tension after quick stretch, it is suggested that there would be another system possessed of time constant of a few seconds than the system detected by sinusoidal analysis.
8. Visco-elastic properties and their changes in contraction process of glycerol-extracted muscle are similar to those of living muscle.
The authors wish to express their thanks to Prof. Hidenobu Mashima for his criticism of their results. Thanks are also due to Dr. Noboru Nishida for his kind suggestion throughout the experiment. This study was supported by grant for Japan Society for the Promotion of Science.

REFERENCES


DYNAMIC VISCO-ELASTIC PROPERTIES OF MUSCLE

Bull. 96: 140-161.